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MARCH, 1938

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LABYRINTHULA ON PACIFIC COAST EEL-GRASS¹

By EDWARD LORRAINE YOUNG III²

Abstract

The labyrinthulan parasite causing the disease of the Atlantic coast *Zostera marina* L. is reported on *Z. marina* from Departure Bay, Nanaimo, B.C. The gross and histological appearance of infected Pacific coast eel-grass are described and are found to be similar to those of the Atlantic coast grass and the parasites, in each case, are found to be identical.

Introduction

One of the *Labyrinthulae* was shown to be the causative agent of the *Zostera marina* wastage in the Atlantic Ocean by Dr. Charles E. Renn in 1934 (1, 2). He studied specimens of infected eel-grass from many parts of the American and European coasts and found that both the symptoms of the disease and the labyrinthulan parasite associated with them remained constant. The leaves of infected beds are readily identified by their splotched and darkly streaked appearance and they are sloughed off shortly after the disintegration of the greater part of their green tissues. The discoloration is due to the disruption of both the epidermal and mesophyll cells. Death is caused apparently by the destruction of the food synthesizing mechanism, as the stems and roots remain unaffected. Dr. Renn examined grass from Departure Bay in September 1934 and again in the early part of 1936, but found neither symptoms of the disease nor *Labyrinthula*. Another shipment of grass from the Pacific coast in June, 1937, was also negative.

Discovery of *Labyrinthula* on Pacific Coast Eel-grass

Collections of *Zostera marina* L. were made at the Departure Bay Biological Station, Nanaimo, B.C. in September, 1936, by Dr. H. T. Güssow and again in July, 1937, by Dr. W. A. Clemens, and were shipped to the writer for examination by Dr. Irene Mounce. *Labyrinthula* was demonstrated conclusively in both shipments. The specimens of grass were preponderantly of the variety *latifolia*. From gross external appearance the disease manifests itself by the same brown and blackened splotched areas on the blades as it does in the Atlantic Ocean infestation. For study the leaves, preserved in a formalin-acetic-alcohol fixative, were embedded in paraffin, sectioned at 10 microns, and stained with a modification of the Staughton technique for

¹ Manuscript received September 25, 1937.

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wood (safranin, carbol-thionin, orange G). With this stain the tissues are easily differentiated: the diseased parts yellow to orange, the healthy blue to purple, and *Labyrinthula* a gray blue. The chloroplasts also stain blue but are more purple and brilliant than the parasite.

Histologically the picture seen here is identical with that observed in Atlantic Ocean *Z. marina*. The healthy normal tissue is bordered by diseased cells. The contents of these latter cells are in various stages of dissolution: the chloroplasts are disrupted; the protoplasm is flocculated and clumped either at the periphery or in the centre of the cell; the cell walls are irregular. Many cells are completely emptied of their contents while others are filled with secondary inclusions such as resins. Often the mesophyll air spaces are occluded by these ergastic substances.

The parasite is most abundant just ahead of the badly diseased tissue either in recently invaded cells or just beyond in apparently still healthy areas. Scattered spindles (cell bodies) of the parasite and resting bodies are found in areas of older infection. The parasite is most clearly demonstrated in the mesophyll air spaces.

The organism present in western eel-grass appears to be identical with that found on the Atlantic grass which has been identified by Young as *Labyrinthula macrocystis* Cienkowski. The cell bodies or spindles are fusiform and average $10 \times 3\mu$. A central vesicular nucleus and nucleolus are visible. These spindles are joined by a filamentous net-track which extends from cell to cell. On this network the spindles glide, boring through the cell walls probably by enzymatic action. The resting bodies are spherical and $6-10\mu$ in diameter. In sum, the pathological picture presented by the *Zostera marina* from Departure Bay is similar in all respects to that of grass from any part of the Atlantic seaboard.

Discussion

It is difficult either to account for the presence of *Labyrinthula* in Pacific coast eel-grass or to foretell what it may mean. The sudden appearance of the disease on the Atlantic coast has not been explained satisfactorily as yet, and so it is equally hard to account for its sudden occurrence at Nanaimo. Oceanographic and climatic data supplied through the courtesy of Dr. W. A. Clemens, Director of the Biological Station at Departure Bay, are being studied in an attempt to find some one or more environmental factors which have changed sufficiently and universally enough to account for its sudden rise. Temperature changes appear inadequate since the water at Departure Bay has increased in mean yearly temperature but 0.8°C . in the last three years (10.9° to 11.7°C .). It may be of more significance that the salinity has increased as it has been found along the Atlantic coast that grass in sheltered areas of reduced salinity appeared more resistant to the disease. For June, 1934 to March 1935, the mean chlorinity percentage at Departure Bay was 13.69; for the next year 14.30; and for this last year 14.72.

On the other hand Renn and Lynch examined plantations of Pacific grass in Great Bay, Long Island, in early June, 1936. These beds were in seed and were beginning to waste away, but without exhibiting the black streaks or splotches characteristic of the labyrinthulan disease, and no *Labyrinthulae* were found on examination. Whether the eel-grass on the Pacific coast is not very susceptible to the disease, whether environmental conditions are not such as to favor the development of an epidemic, whether the parasite may remain endemic in Departure Bay, or whether the western eel-grass too may be wiped out remains at present a matter of conjecture.

Acknowledgments

The author is much indebted to Dr. Irene Mounce for supplying the grass from Departure Bay and for her interest and help in preparing this paper; to Dr. C. E. Renn, Dr. W. A. Clemens, and Dr. H. T. Güssow for the information and specimens which they furnished.

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YELLOW BLOTCH-CURL: A NEW VIRUS DISEASE OF THE RED RASPBERRY IN ONTARIO¹

By G. C. CHAMBERLAIN²

Abstract

This paper reports the results of an investigation into a condition of the Cuthbert red raspberry which is quite distinct from any previously described disease. The disease, termed yellow blotch-curl, is characterized principally by a loose type of curling and pale chlorotic foliage which sometimes shows a yellow blotching and ring spot. The disease has been transmitted by patch-grafting, which indicates that it is of the virus type. It has been transmitted to Cuthbert, Viking, Latham, Herbert, Chief, and Lloyd George varieties, and different reactions have been noted and described.

From these experiments it is concluded that "yellow blotch-curl" is distinct from mosaic and leaf curl.

Introduction

In 1935 a general investigation of the virus diseases of the red raspberry was undertaken in order to compare varietal reactions to the mosaics and leaf curl, and to study the possibility of virus infection in any plants showing unusual symptoms. During the latter phase of this work, two new virus diseases were encountered, one of which is dealt with in the present paper. This was found in a Cuthbert plantation where a number of plants at one end of the nursery rows showed a decided lack of vigor accompanied by an unusual arching and curling of the foliage. This condition, which had been observed in other plantings, has hitherto been ascribed to some unfavorable soil condition. The possibility of virus infection, however, was suggested by the presence on one of the canes of several basal leaflets exhibiting distinct yellow blotches or spots, some of which were in the form of rings. This cane was brought to the laboratory for use as a source of scion material for transmission experiments by grafting. These experiments have been successful in determining the cause to be virus infection. Because of the character of the symptoms, the name "yellow blotch-curl" is suggested.

Occurrence

Undoubtedly this disease has been present in Ontario for a number of years, although it was not recognized as such until 1935. It has been observed repeatedly as an important factor in the variety Cuthbert, but has not been recognized in other varieties, except as the result of grafting. In 1937, all plantings visited were critically inspected, with the result that in five of seven nurseries and six of eleven commercial plantings the disease could be readily found. In one instance, as a result of investigating a complaint regarding the failure of a five-year-old three-acre plantation it was found that 90% of the stools were seriously affected. This plantation, though in good fertile gravely

¹ Manuscript received December 29, 1937.

Contribution No. 536 from the Division of Botany, Experimental Farms Branch, Dominion Department of Agriculture, Ottawa, Ontario.

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soil and given excellent care, had steadily declined in production until it was no longer profitable and was destroyed. Since there was but a trace of mosaic and leaf curl present and other factors were favorable for production, the failure could only be ascribed to the effect of yellow blotch-curl disease.

Symptoms on Cuthbert in the Field

A noticeable effect of this disease is the reduction in number of canes and a remarkable dwarfing and stunting of growth. Affected stools stand out in contrast to normal ones and appear thin, spindly and lacking vigor. The appearance of such stools is very suggestive of the effect expected from a depleted soil or lack of drainage. The canes exhibit an erect, stiff type of growth and bear pale, chlorotic lustreless foliage. Owing to a shortening of internodes, particularly in the apical regions of the cane, the leaflets appear to cluster. This clustering is accentuated by a loose type of curling in which the leaves become arched and the tips curl down and inwards (Plate I, Fig. 1). The leaf texture also becomes such that a noticeable rattle is heard when the canes are shaken.

While the above symptoms are characteristic and commonly encountered, canes may also be found on which a few of the older basal leaflets exhibit a coarse, yellow blotching and spotting, often as definite ring spots. Frequently, however, these symptoms do not occur.

Materials and Methods

Experimental Work

The patch-grafting method, used for transmitting the virus, consists of replacing a patch of bark from a stock plant with a similar one from a scion plant. A special set of razor-blade knives, originated by and obtained from W. A. Roach (6) was used to make patches of equal size. After the graft was made, it was wrapped for a week with a crepe rubber bandage known as Sterilastic. It was later found desirable to cover the wound first with a piece of waxed paper to prevent the bandage adhering to the patch. The varieties used for grafting included Cuthbert, Viking, Latham, Herbert, Chief and Lloyd George, selected because of their apparent differences in regard to klendusity and susceptibility to mosaic (5). With the exception of Latham, which were specially selected from known healthy stools, all the plants were of virus-free clonal origin and were set in the field eight feet apart each way and received good cultural attention. Care was taken to see that the suckers were confined to the immediate vicinity of the parent plant. The grafts were generally placed low on the canes, although any area where the cane was firm enough not to break at the cut proved satisfactory.

The results of these transmission experiments carried on over a period of three years, clearly show that yellow blotch-curl has been repeatedly transmitted by patch grafting and that it can be passed to varieties other than Cuthbert, on which it was first found. On the other hand, attempts to transmit the disease to black raspberries have so far resulted in failure.

The first indication of transmission was noted September 21, 1935, on one of the two Cuthbert canes grafted July 12. A clustering and curling of the leaflets accompanied by a coarse yellow blotchiness that appeared at the tip of a late lateral were similar to the symptoms on the scion cane. By the following spring, both grafted stools were systemically infected and showed identical symptoms typical of those noted in the field. These two stools therefore were used as sources of scion material for many of the later grafts which verified the transmissibility and distinctive character of this disease.

TABLE I

RESULTS OF EXPERIMENTS TO TRANSMIT YELLOW BLOTCH-CURL BY PATCH GRAFTING, 1935-37

Affected scion	Healthy stock	Date	No. of grafts	Transmission	
				Positive	Negative
Cuthbert*	Viking	12.7.35	1	0	1
Cuthbert*	Cuthbert	12.7.35	2	2	0
Cuthbert	Cuthbert	2.6.36	2	2	0
Cuthbert	Viking	2.6.36	3	3	0
Cuthbert	Latham	12.6.36	8	7	1
Cuthbert	Viking	3.7.36	2	0	2
Cuthbert	Cuthbert	3.7.36	2	1	1
Cuthbert	Chief	3.7.36	2	2	0
Cuthbert	Viking	21.8.36	3	3	0
Cuthbert*	Viking	21.8.36	2	2	0
Cuthbert	Cuthbert	21.8.36	3	2	1
Cuthbert	Latham	21.8.36	4	3	1
Cuthbert	L. George	21.8.36	2	1	1
Cuthbert	Herbert	21.8.36	3	3	0
Cuthbert	Viking	22.9.36	3	0	3
Cuthbert	Bl. Raspberry	11.6.37	9	0	9
Latham	Viking	25.6.37	2	2	0
Viking	Viking	25.6.37	5	4	1
Cuthbert	Viking	25.6.37	4	4	0
Latham	Cuthbert	25.6.37	2	1	1
Latham	Cuthbert	25.6.37	4	3	1
Cuthbert	Cuthbert	25.6.37	4	4	0
Cuthbert	Viking	7.7.37	5	4	1
Chief	Viking	7.7.37	2	0	2
Cuthbert	Cuthbert	7.7.37	4	3	1
Cuthbert*	Viking	14.7.37	4	0	4
Cuthbert*	Viking	17.7.37	4	1	3
Cuthbert*	Cuthbert	17.7.37	4	3	1
Cuthbert*	Viking	6.8.37	3	0	3
Cuthbert*	Cuthbert	6.8.37	3	0	3
Cuthbert*	Cuthbert	7.8.37	4	0	4
Cuthbert*	Viking	7.8.37	4	0	4
Totals			109	60	49

* Scion material suspected of being infected with yellow blotch-curl.

Yellow blotch-curl has been transmitted not only from Cuthbert to Cuthbert, but also from Cuthbert to Latham, Herbert, Chief, Lloyd George and Viking. It has been transferred back to Cuthbert from Latham and also

TABLE II
SUMMARY OF EXPERIMENTS ON VARIETAL REACTION OF RASPBERRIES TO YELLOW BLOTCH-CURL

Mosaic				Yellow blotch-curl							
Variety	Klendusity	Susceptibility	Degree of symptom-expression	Effect or reaction	Symptom expression				Effect on growth		Remarks
					Stunting effect	Yellow blotch	Ring spot	Curling	Suckers	Vigor	
Latham	++	+++	Masked	+++	+++	+++	0	+++	0 - +	Poor	Cane died
Herbert	+++	+++	Pronounced	+++	+++	+++	0	+++	0 - +	Poor	Cane died
Chief	+++	+	Highly masked	+	+	+	+	+++	+++	Fair	Weak cane growth
L. George	+++	++	Fairly pronounced	+++	+++	++	0	+++	+	Poor	Cane died
Viking	+	+++	Pronounced	+++	+++	+++	++	+++	+	Poor	Weakened cane growth
Cuthbert	+	+++	Pronounced	+	+	+++	+++	+++	+++	Fair	Cane growth reduced

The + sign is used to denote the following: + low or slight; ++ moderate; +++ high or severe; ++++ very severe.
Under "Effect on growth" of suckers: 0 absent; + very limited; ++ limited; +++ average production.

from Viking to Viking. As with other virus diseases, different varietal reactions to yellow blotch-curl have been noted. This is indicated in Table II.

It is evident from Table II that the six varieties may be divided into three main groups according to their reaction to yellow blotch-curl infection. Latham and Herbert proved distinctly susceptible, the fruiting canes dying after a weak, stunted growth of laterals bearing small, tightly-curled leaves (Plate I, Fig. 2). In the majority of instances the stools were completely killed and suckers either failed to appear or died following a poor stunted growth. Chief and Cuthbert form a group, far less seriously affected, especially as regards the stooling ability of the plants. The fruiting canes showed the usual delay in foliation and a general weakened growth of laterals, and while the sucker production was reduced especially, in Chief, which is normally prolific in this regard, still a number developed and made fair growth. Lloyd George and Viking may be placed in an intermediate group in degree of susceptibility, even though the effect on the growth of the stool was drastic. The Lloyd George fruiting canes died prematurely, but unlike the Latham and Herbert, the stool produced a limited number of suckers which continued to produce a weak growth. The Viking canes in most cases produced a weak growth of fruiting laterals (Plate II, Fig. 2) and a few worthless, small, unevenly ripened and misshapen berries. New cane production was *nil* or very limited and poor in vigor and growth.

The chlorotic or yellow blotch and spot symptoms were always more or less evident on grafted plants. It was, however, found to vary in occurrence and definition on the different varieties. On Latham, Viking and Cuthbert, for instance, it was not only more common but more pronounced (Plate II, Figs. 1 and 3). Very often the blotching was extensive, involving the entire lamina; in other cases it was more limited and confined to areas along the main veins or at the leaf margin. Sometimes it occurred as definite spots of varying size either singly or in groups. There was no mottling associated with this symptom nor was it accompanied by blistering or puckering of the leaf surface, although an asymmetrical distortion sometimes followed the occurrence of the blotches about main veins or at the leaf margin. In Cuthbert the blotching occasionally took the form of a yellow pattern. In general, these symptoms were confined to the older or early formed leaves, although at times they would appear in distinct zones similar to mosaic mottling. On both Lloyd George and Chief this symptom was suppressed. In Chief, particularly, the blotching was indistinct and appeared more as a paling of color and without definite character. The ring spots associated with the blotch symptom appeared on only three varieties, commonly on Cuthbert, infrequently on Viking, and only occasionally on Chief. On the latter, the rings were faint and bordered with a pale green, rather than a pronounced yellow as in the others.

The curling of the foliage was a symptom character common to all the varieties. On the fruiting canes of Latham, Herbert and Viking, a decided

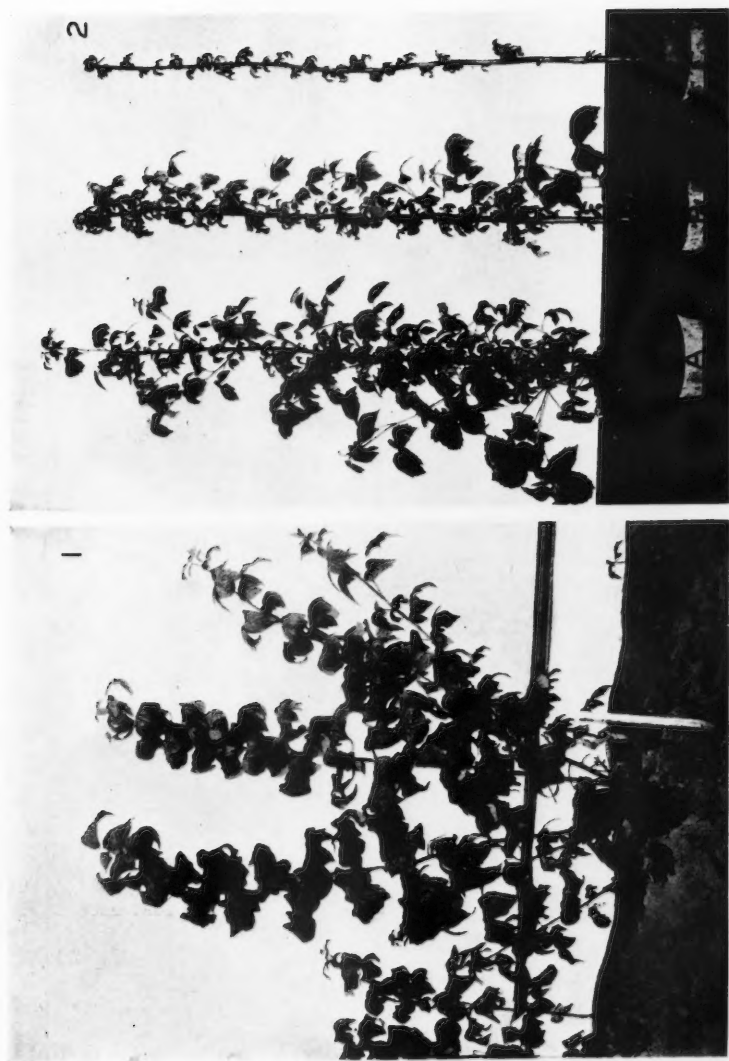


FIG. 1. A Cuthbert stool affected with yellow blotch-curl. Note clustering and curling of leaflets. Grafted August 21, 1936. Photographed August 26, 1937. FIG. 2. Three Latham fruiting canes affected with yellow blotch-curl, in varying degrees. A, slightly affected; B, disease not entirely systemic, all laterals on one side of cane seriously affected; C, severe effect; the stunting of laterals in C is very drastic. Leaves show extensive yellow blotches. From stools grafted June 12, 1936. Photographed June 3, 1937.

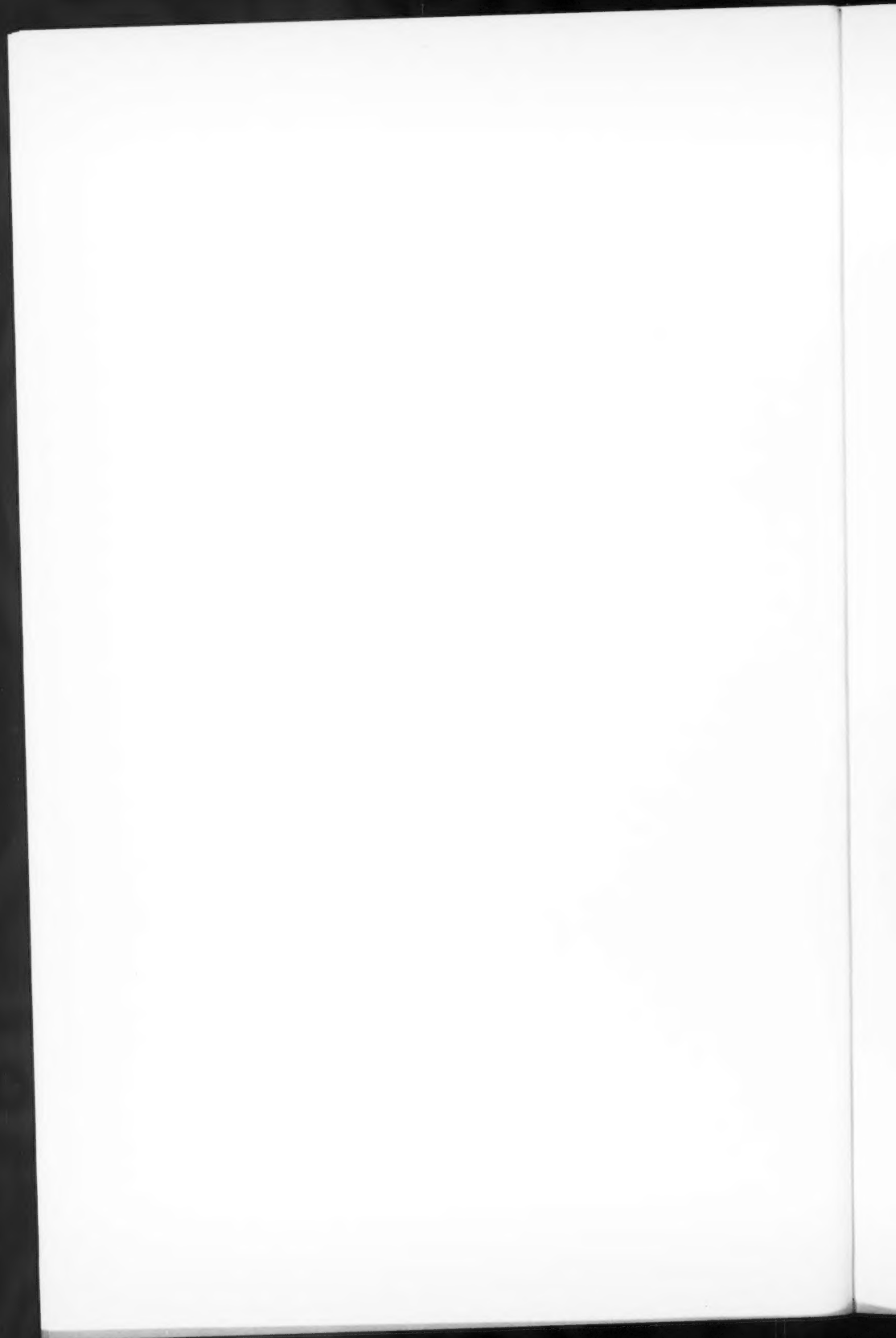


PLATE II

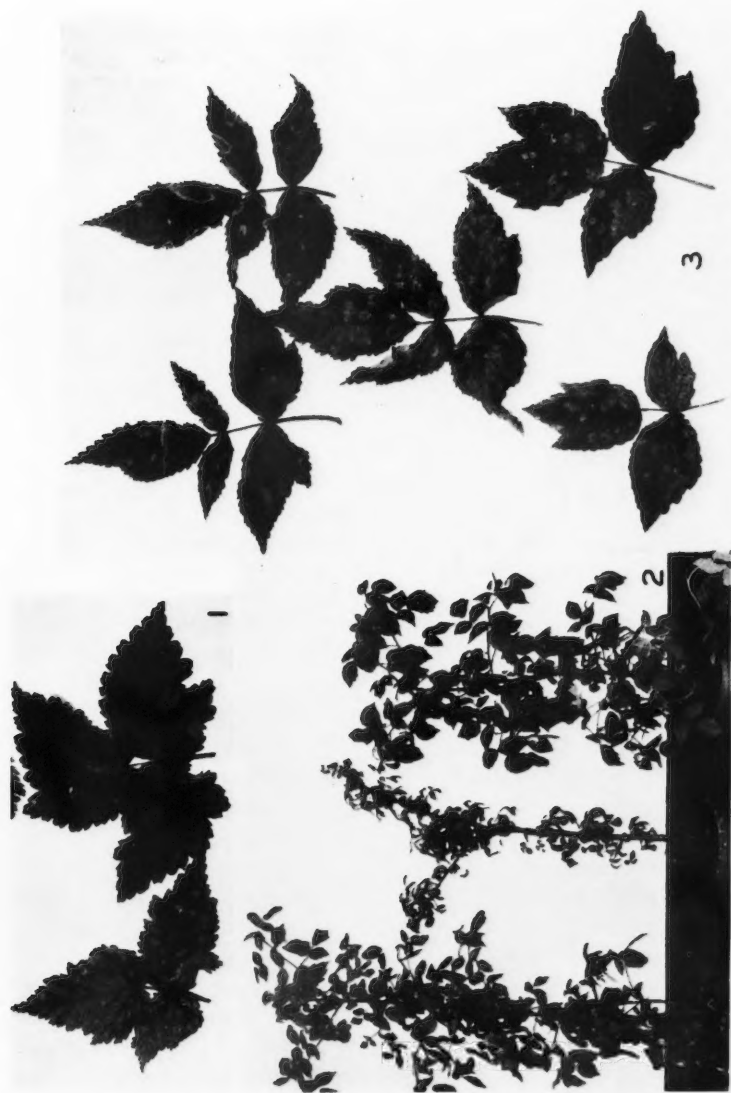
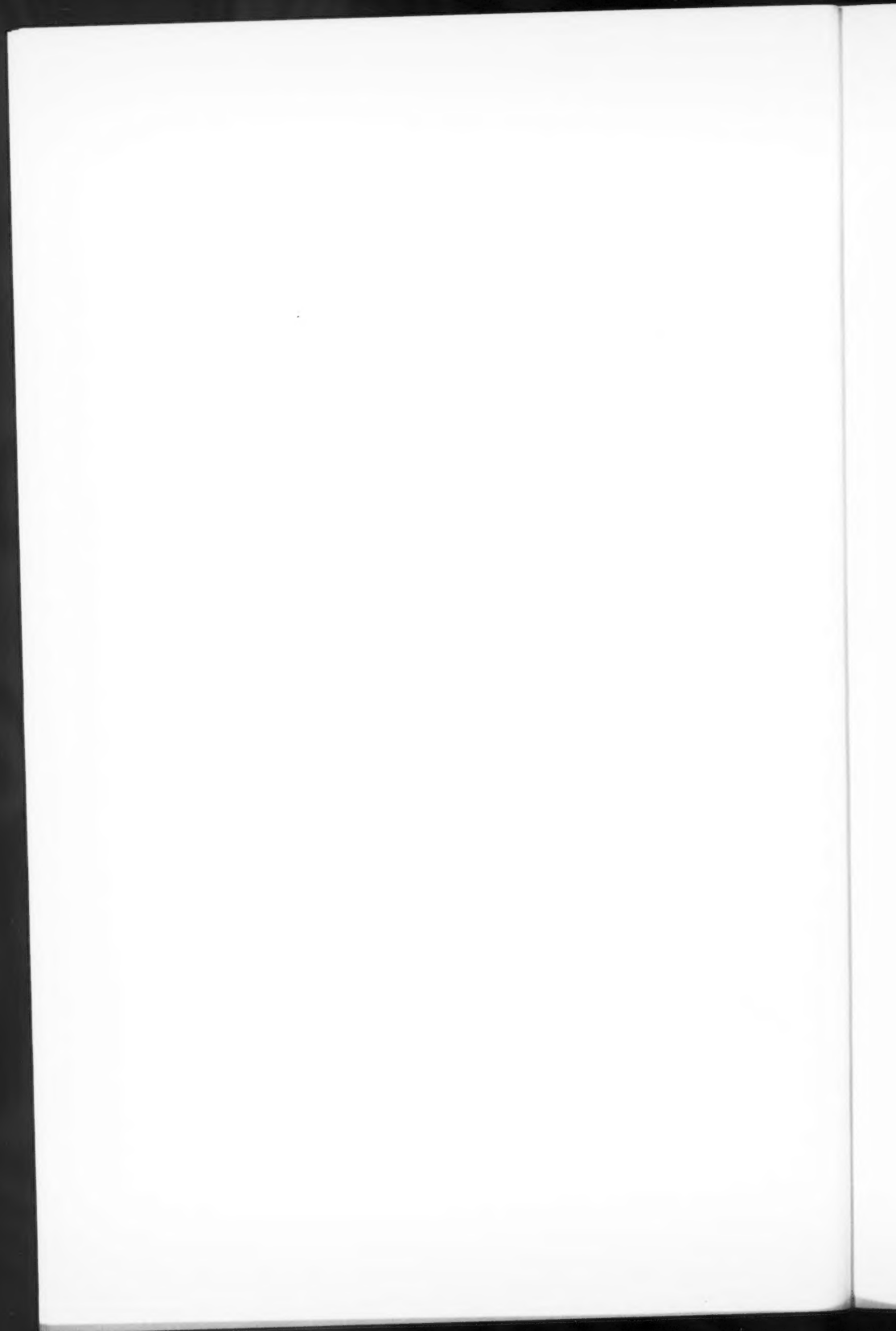


FIG. 1. Leaflets from a Viking cane affected with yellow blotch-curl showing blotching and spotting of the leaf.
 FIG. 2. The effect of yellow blotch-curl on fruiting cane of Viking, centre; compared with mosaic, left, and healthy cane, right.
 Grafted August 21, 1936. Photographed June 3, 1937. FIG. 3. Leaflets of Cubbert showing blotching, spotting and
 ring spot symptom of yellow blotch-curl common on grafted plants. From plant grafted June 2, 1936. Photographed
 September 18, 1936.



curl accompanied by dwarfing of foliage developed. On the new canes of these varieties it was less drastic, but it was characteristic for the growth to cease when the tips became curled. On the other hand the new canes of the other varieties continued to grow and showed a clustering effect of leaflets and a loose curling of the foliage, the symptom commonly encountered in the field (Plate I, Fig. 1).

Discussion

In all cases where transmission occurred it was noted that infection was not systemic until the second year. Symptoms appeared first, not on the central leader of the grafted cane, but on one or more lateral branches and most frequently on those arising nearest the graft. With varieties such as Latham, which form few branches, the first symptoms show on a few of the suckers and not on the original grafted cane except as the disease becomes systemic the following year. This was also found to be true in cases where mosaic scion material was used for grafting.

June or early July proved to be the most favorable time for grafting. At this time growth is succulent, with the result that scion and stock patches strip clean and are easily handled. In addition, the results obtained indicate that the incubation period for yellow blotch-curl may vary considerably and is quite lengthy, and, therefore, unless grafts are made in June or early July, symptoms do not appear on the current season's growth. There was also evidence to indicate that the incubation period may be shortened if vigorous, rapidly growing plants are inoculated. This was demonstrated in 1937 when a more favorable growing season made earlier grafting possible. In one graft a period of 45 days elapsed between the time of grafting and symptom expression, compared to more than 70 days in 1936.

The relation of early grafting to symptom expression in the current season may be mentioned in connection with the grafts made in August, 1937. These grafts are recorded in Table I as negative so far as transmission is concerned. However, the results are as yet incomplete, as previous experience suggests the grafts were made too late in the season for symptoms to appear.

The two virus diseases, mosaic and leaf curl, have been of importance in Ontario for the past 25 years. Mosaic is manifested by a general mottling, accompanied by blistering and puckering of the foliage. The symptoms of leaf curl are a severe tight curling of the foliage which is dark green and appears greasy (3). Both these diseases have been transmitted by patch grafting with resulting symptoms typical for each disease. The symptoms as described above for yellow blotch-curl are therefore sufficiently distinct from either mosaic or leaf curl to warrant considering yellow blotch-curl as a new virus disease. While no definite survey has as yet been made to determine the importance of this disease, it is felt from a limited experience that it may be of more importance than is at present realized.

Acknowledgment

The author wishes to express his indebtedness to Dr. G. H. Berkeley, Senior Officer-in-charge of the Dominion Laboratory of Plant Pathology at St. Catharines, Ontario, for his direction and help during the course of the work.

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STUDIES ON FOOT AND ROOT ROT OF WHEAT

V. THE RELATION OF PHOSPHORUS, POTASSIUM, NITROGEN, AND CALCIUM NUTRITION TO THE FOOT- AND ROOT-ROT DISEASE OF WHEAT CAUSED BY *HELMINTHOSPORIUM SATIVUM* P. K. & B.¹

BY W. C. BROADFOOT² AND L. E. TYNER³

Abstract

The effect of different amounts of phosphorus, potassium, nitrogen, and calcium upon the development of the foot-rot disease of wheat caused by *Helminthosporium sativum* P. K. & B. was studied in the greenhouse. The wheat grains were planted in sterilized pure quartz sand to which the necessary nutrients and spore suspension of the pathogen were added. The experiments were maintained under aseptic conditions during the first ten days. The disease increased when the ionic concentration of potassium, nitrogen, and calcium was decreased below that of the complete nutrient solution, but no significant reduction of the disease was observed when the concentrations of all of the elements, including phosphorus, were increased above those in the complete nutrient solution. Apparently extremely small concentrations of phosphorus had no effect on the disease one way or the other. These conclusions apply to the disease on the seedling stage of wheat.

Of the many food and other growth factors which contribute to the general health of the wheat plant, probably none is more important than mineral nutrition. The symptoms of nitrogen, phosphorus and potassium starvation of this plant, and the remedy for it, are well known and fully described in a number of books. An extensive review of the literature relating to the factors affecting the absorption of these essential elements by plants has been made by Hoagland (2). There is also the direct role of a deficiency of certain minor elements in producing pathological conditions, which has been discussed by Jacks and Scherbatoff (3).

In addition to the direct effect on plant growth of the minerals referred to above, a deficiency or excess of one or more of these might render a host more susceptible to certain disease-producing micro-organisms than it would otherwise be. This premise would seem to have special application to the foot- and root-rot diseases of cereals, since both host and pathogen must develop in the same soil habitat where the metabolism of each is undoubtedly affected by the kind of minerals available. No reference, however, has been found in the literature concerning the effect of the absorption of varying amounts of nitrogen, phosphorus, potassium, and calcium on the ability of the wheat plant to withstand infection and injury by *Helminthosporium sativum* P. K. & B.

Since our early field experiments on mineral nutrition gave extremely variable results, it became necessary to transfer the study to the greenhouse,

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³ Plant Disease Investigator, Dominion Laboratory of Plant Pathology, Edmonton, Canada.

where the chemical and other usual environmental factors could be better controlled, and also where a more precise experimental technique could be employed. The present greenhouse investigation has been principally concerned with the nutrition of the wheat plant as a factor in its pre-disposition to attack by *H. sativum*. It has involved a study of the development of the foot and root rot of wheat caused by this pathogen in known ionic concentrations of nitrogen, phosphorus, potassium, and calcium as added to a silica sand substrate. Observations were also made on the growth of the pathogen itself in pure culture solutions to which the minerals mentioned were added.

Materials and Methods

The nutrient solutions employed were varied according to need from basic formulas which were kindly furnished by Professor D. R. Hoagland, of the University of California. His complete solution (Table I) was used as a basis for comparison in all experiments. The required chemicals, in C.P. form, were added to wide-mouth, one-litre flasks, each containing one-half kilogram of pure silica sand, obtained from Ottawa, Illinois. The flasks, with cotton plugs, were autoclaved at 20 lb. pressure for 6 hr. Each flask was aseptically seeded with 30 grains of Elite Marquis wheat, which had been previously soaked in water for 3 hr. at 20° C. and surface disinfected in 1-1000 HgCl₂ for 20 min. The planted seeds were drenched with a spore suspension of a virulent one-month-old culture of *H. sativum*, and were then covered with 250 cc. of sterilized silica sand. In the control series, the spore suspension was replaced by an equal volume of sterile distilled water. The flasks were weighed at weekly intervals and the sand was maintained at 70% of its moisture-holding capacity by adding distilled water containing 1 cc. per litre of 0.5% ferric tartrate. At the end of 40 days, the number of plants, the height, and the infection rating were recorded. The plants were dried for 48 hr. in an oven at 90° C., and then weighed. The following values for infection were assigned: 0, clean; 1, trace; 2, light-; 3, light; 4, light+; 5, medium-; 6, medium; 7, medium+; 8, heavy-; 9, heavy; 10, heavy+. Four experiments for the silica sand series are reported, viz.: Nos. 1, 2, 3, and 8.

The results obtained from growing the pathogen in pure culture, in the same nutrient solutions as were used in the corresponding silica sand experiments, are presented separately. Other necessary details of technique will be supplied in conjunction with each experiment. The experimental data were tested by Fisher's (1) Analysis of Variance method, and the "F" test of Snedecor (4), to determine the significance of the differences observed between the various treatments.

Experimental Results

EXPERIMENT I

Effect of Omission and of Excess of P, K, N, and Ca

In this experiment the effect of the omission and also of the excess of phosphorus, potassium, nitrogen and calcium ions upon the development of disease

was investigated. The composition of the nutrient solutions is given in Table I. Each nutrient solution in both infested and control series was replicated ten times. This experiment was taken up on November 7, 1933.

An examination of the data in Table II shows that the F value for nutrients exceeded the 1% point for number, height, and infection rating in the infested series. It is quite evident that, for each of the nutrients, the plants in the infested series were shorter and lighter than the plants in the control series (see Plate I). Disease development was most severe in the solutions deficient

TABLE I
COMPOSITION OF NUTRIENT SOLUTIONS (CC. PER LITRE) EMPLOYED IN EXPERIMENT I

Nutrient solutions	Ca(NO ₃) ₂	KNO ₃	MgSO ₄	KH ₂ PO ₄	K ₂ SO ₄	Ca(H ₂ PO ₄) ₂	H ₂ PO ₄	KCl	NH ₄ NO ₃	CaCl ₂
	M/1	M/1	M/1	M/1	M/.5	M/.01	M/1	M/1	M/1	M/1
1 H ₂ O (dist.)										
2 Complete	5	5	2	1						
3 Minus P	7.5		2		10					
4 Excess P	7.5		2	1		50	5			
5 Minus K	7.5		2			50				
6 Excess K	5	5	2	1	10			5		
7 Minus N			2		10	50				
8 Excess N	8	5	2			50			5	
9 Minus Ca		10	2	1						
10 Excess Ca	7.5		2		10	50				5

TABLE II
EFFECT OF THE OMISSION AND THE EXCESS OF PHOSPHORUS, POTASSIUM, NITROGEN, AND CALCIUM UPON THE NUMBER, HEIGHT, INFECTION RATING, AND WEIGHT, OF MARQUIS WHEAT SEEDLINGS GROWN IN SILICA SAND, INFESTED WITH *Helminthosporium sativum*

Nutrient solutions	Number of plants		Height, cm.		Infection rating	Weight, mg.	
	Infested series	Control	Infested series	Control		Infested series	Control
1 H ₂ O (dist.)	22.9—	26.5	19.9	25.4—	8.0+	21.4	29.2
2 Complete	25.3	28.9	30.1	35.6	5.6	46.7	61.3
3 Minus P	27.9+	26.9	30.0	36.1	4.4—	56.3	58.6
4 Excess P	26.9	27.6	29.9	34.5—	4.4—	48.3	58.8
5 Minus K	23.3	22.9—	16.0—	19.1—	8.8+	24.0	21.3
6 Excess K	26.6	23.4—	28.9	32.0—	4.0—	64.3	65.8
7 Minus N	28.0+	28.3	20.1—	24.0—	6.4+	35.0	36.7
8 Excess N	26.0	28.3	29.5	34.4—	5.0	60.8	65.9
9 Minus Ca	24.7	24.6—	18.4—	21.6—	8.8+	22.3	27.4
10 Excess Ca	28.0+	27.0	31.8+	34.6—	4.5—	63.2	74.5
2 X $\sqrt{2}$ X S.E.	2.1	2.5	1.0	0.9	0.5		
"F" nutrients	6.24	5.66	307.34	512.67	108.99		
1% point	2.74	2.82	2.74	2.82	2.74		
"F" replicates	.78	.38	8.55	15.00	1.01		
5% point	2.06	2.25	2.06	2.25	2.06		

+ and — indicate that these values are significantly higher or lower than those for the complete solution.

in potassium, nitrogen and calcium. Although it would appear that disease development was less on the plants in the solutions with excess phosphorus, potassium, nitrogen and calcium than in the complete nutrient solution, quantitative data, such as height, suggest that there is no marked difference. The error due to the difficulty of washing out all of the silica sand from the roots vitiated the reliability of the data on the dry weight of the plants. It was apparent, from the growth of the plants obtained in the phosphorus-deficient series that sufficient phosphorus was provided by the seed or by impurities in either the C.P. chemicals or the silica sand used.

EXPERIMENT II

Effect of Deficiency and of Excess of P, K, N, and Ca

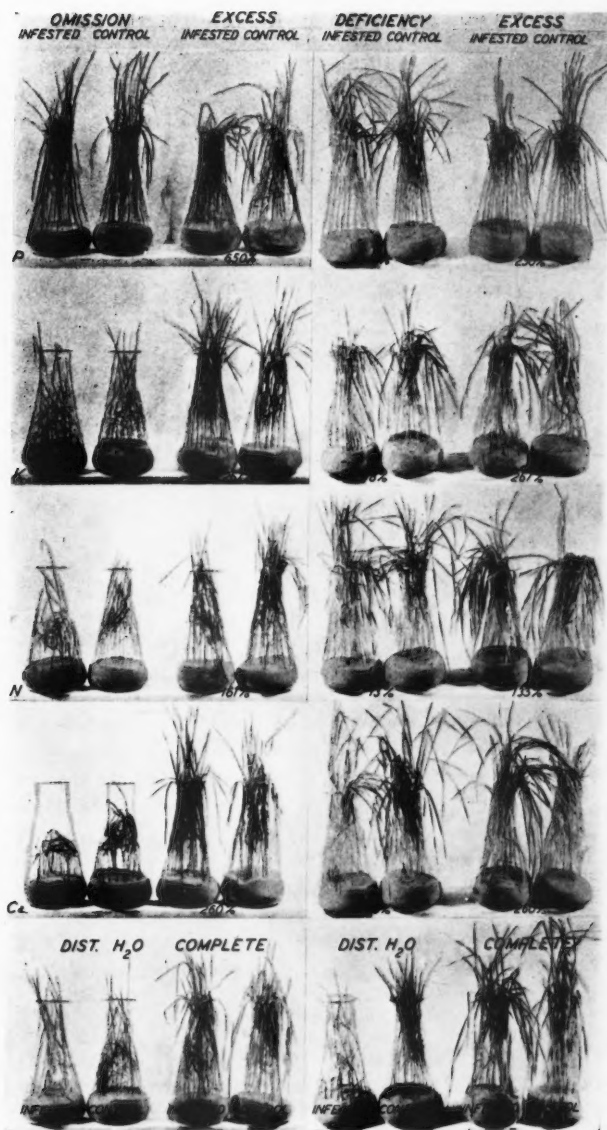
In Experiment I, very poor growth was obtained in the series where potassium, nitrogen and calcium ions were omitted from the solutions. Therefore, in this experiment the effect of a deficient amount of phosphorus, potassium, nitrogen and calcium ions, and also of an excess of them, upon the development of disease was observed. The composition of the nutrient solutions is given in Table III. Each nutrient solution was replicated ten times in the infested series, and five times in the control series. This experiment was taken up on February 9, 1934.

As in Experiment I, the F value of the effect of the nutrients exceeded the 1% point for number, height, and infection rating in the series containing the pathogen, and for height in the series where the pathogen was omitted. The plants in the infested series were shorter than those with the same nutrient solution in the control series (Table IV, Plate I). Although the development of disease was more severe in the potassium-, nitrogen- and calcium-deficient series than in the excess series, it was, nevertheless, not as severe as it was in Experiment I, where these ions were entirely omitted. In other words, it appears that in the presence of a restricted supply of the ions, the incidence of the disease was more severe than in the complete culture, but not as severe as in the cultures from which they were omitted entirely.

TABLE III
COMPOSITION OF NUTRIENT SOLUTIONS (CC. PER LITRE) EMPLOYED IN EXPERIMENT II

Nutrient solutions	Ca(NO ₃) ₂	KNO ₃	MgSO ₄	KH ₂ PO ₄	K ₂ SO ₄	Ca(H ₂ PO ₄) ₂	H ₂ PO ₄	KCl	NH ₄ NO ₃	CaCl ₂
	M/1	M/1	M/1	M/.1	M/.5	M/.01	M/1	M/1	M/1	M/1
1 H ₂ O (dist.)										
2 Complete	5	5	2	1						
3 Minus P	5		2		10					
4 Excess P	7.5		2	1		50	1			
5 8% K	7.5		2		1	50				
6 Excess K	2	5	2	1	10			5		
7 13% N	1		2		10	50				
8 Excess N	5	5	2			50			5	
9 20% Ca	1	10	2	1						
10 Excess Ca	7.5		2		10	50				5

PLATE I



Effect of the omission, deficiency, and excess of phosphorus, potassium, nitrogen, and calcium upon the development of foot rot of Marquis wheat seedlings grown in silica sand, infested with *Helminthosporium salivum*.

TABLE IV

EFFECT OF THE DEFICIENCY AND THE EXCESS OF PHOSPHORUS, POTASSIUM, NITROGEN, AND CALCIUM UPON THE NUMBER, HEIGHT, INFECTION RATING AND WEIGHT OF MARQUIS WHEAT SEEDLINGS GROWN IN SILICA SAND, INFESTED WITH *Helminthosporium sativum*

Nutrient solutions	Number of plants		Height, cm.		Infection rating	Weight, mg.	
	Infested series	Control	Infested series	Control		Infested series	Control
1 H ₂ O (dist.)	21.6 -	26.2 -	16.8 -	23.0 -	9.2 +	20.9	26.0
2 Complete	27.1	28.8	39.8	45.0	5.2	43.9	67.9
3 Minus P	27.7	28.2	38.1	44.0	5.4	59.2	71.7
4 Excess P	27.8	28.0	37.7 -	42.0	4.9	55.4	57.9
5 8% K	27.0	28.6	38.6	38.8 -	7.0 +	58.1	60.1
6 Excess K	24.9 -	26.8 -	34.5 -	42.8	4.8 -	47.2	51.5
7 13% N	25.8	28.4	32.9 -	43.0	5.8 +	48.8	51.4
8 Excess N	26.8	29.2	36.3 -	45.0	5.1	47.4	66.4
9 20% Ca	26.7	28.8	37.0 -	41.5 -	5.9 +	47.6	58.3
10 Excess Ca	26.0	28.2	40.3	44.0	4.6 -	61.2	58.9
2 X $\sqrt{2}$ X S.E.	1.9	1.7	.9	3.0	.4		
"F" nutrients	7.26	2.50	118.68	37.26	128.51		
1% point	2.74	3.07	2.74	3.07	2.74		
"F" replicates	1.43	.47	.65	.66	1.39		
5% point	2.06	2.64	2.06	2.64	2.06		

+ and - indicate that these values are significantly higher or lower than those for the complete solution.

EXPERIMENT III

Effect of Decreasing the Concentration of K, N, and Ca

The addition of small amounts of potassium, nitrogen and calcium ions in Experiment II reduced the disease markedly. Therefore, the effect of varying, within narrow limits, the concentration of these ions upon the development of the disease was studied in this experiment. The composition of the nutrient solutions is given in Table V. Each nutrient solution was replicated five times. This experiment was taken up on April 7, 1934.

The experimental data given in Table VI, and illustrated in Fig. 1, verified the results of Experiment II, which indicated the necessity of having at least minimum amounts of these three ions (K, N, and Ca). The F value for nutrients exceeded the 1% point for number, height, and infection rating. Thus, by decreasing the ionic concentration of K, N, and Ca the disease was increased, and also the height and weight of the plants were decreased.

EXPERIMENT VIII

Effect of Increasing the Concentration of P, K, N, and Ca

Since sub-optimal amounts of potassium, nitrogen and calcium materially affected the metabolism of the wheat plant, as expressed in the data given, it seemed that by increasing the ionic concentration, the disease might be reduced. Using the complete nutrient solution as a base, 2, 4, 6, 8 and 10 cc. per litre of a molar solution of NaH₂PO₄, KCl, NH₄Cl, and CaCl₂ was

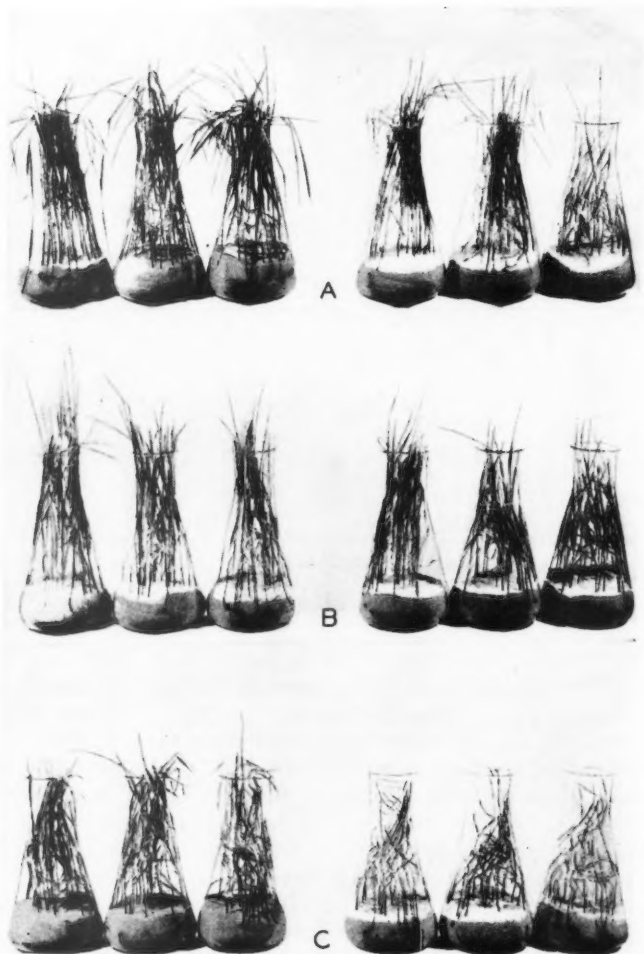


FIG. 1. Effect of decreasing the ionic concentration of potassium, nitrogen, and calcium upon the development of foot rot of Marquis wheat seedlings grown in silica sand, infested with *Helminthosporium sativum*. Left to right: A—17% K, 13% K, 9% K, 4% K, 2% K, minus K. B—13% N, 10% N, 7% N, 3% N, 1% N, minus N. C—20% Ca, 15% Ca, 10% Ca, 5% Ca, 2% Ca, minus Ca.

added to give nutrient solutions of increasing concentrations of phosphorus, potassium, nitrogen and calcium, respectively. Each nutrient was replicated five times. This experiment was taken up February 20, 1935.

The experimental results given in Table VII indicate that, according to the criteria used in these experiments, the increasing of the concentration of

TABLE V
COMPOSITION OF NUTRIENT SOLUTIONS (CC. PER LITRE) EMPLOYED IN EXPERIMENT III

Nutrient solutions	Ca(NO ₃) ₂ M/1	KNO ₃ M/1	MgSO ₄ M/1	KH ₂ PO ₄ M/1	Ca(H ₂ PO ₄) ₂ M/.01	K ₂ SO ₄ M/.5
1 H ₂ O (dist.)						
2 Complete	5	5	2	1		
3 17% K	7.5	1	2		50	
4 13% K	7.5	.75	2		50	
5 9% K	7.5	.50	2		50	
6 4% K	7.5	.25	2		50	
7 2% K	7.5	.10	2		50	
8 Minus K	7.5		2		50	
9 13% N	1.0		2		50	10
10 10% N	.75		2		50	10
11 7% N	.50		2		50	10
12 3% N	.25		2		50	10
13 1% N	.10		2		50	10
14 Minus N			2		50	10
15 20% Ca	1.0	10	2	1		
16 15% Ca	.75	10	2	1		
17 10% Ca	.50	10	2	1		
18 5% Ca	.25	10	2	1		
19 2% Ca	.10	10	2	1		
20 Minus Ca		10	2	1		

TABLE VI
EFFECT OF DECREASING THE IONIC CONCENTRATION OF POTASSIUM, NITROGEN AND CALCIUM UPON THE NUMBER, HEIGHT, INFECTION RATING, AND WEIGHT OF MARQUIS WHEAT SEEDLINGS GROWN IN SILICA SAND, INFESTED WITH *Helminthosporium sativum*

Nutrient solutions	Number of plants	Height, cm.	Infection rating	Weight, mg.
1 H ₂ O (dist.)	22.2—	18.1—	8.6+	36.0
2 Complete	28.6	30.1	5.2	125.2
3 17% K	28.0	31.1	5.7	118.6
4 13% K	26.8	32.4+	6.0+	131.3
5 9% K	27.8	29.3	6.3+	107.9
6 4% K	27.4	30.0	6.6+	75.9
7 2% K	26.4	26.4—	6.9+	65.9
8 Minus K	25.6—	19.8—	8.1+	40.6
9 13% N	26.2	28.5—	5.1	77.9
10 10% N	27.6	25.1—	5.8+	73.9
11 7% N	26.6	22.2—	6.4+	60.2
12 3% N	27.0	21.3—	6.9+	52.6
13 1% N	27.6	18.3—	7.5+	51.4
14 Minus N	27.0	18.8—	7.6+	47.4
15 20% Ca	26.2	26.2—	7.0+	64.9
16 15% Ca	25.4—	24.1—	6.8+	59.1
17 10% Ca	25.6—	20.2—	8.0+	42.2
18 5% Ca	26.6	17.8—	8.8+	46.6
19 2% Ca	26.2	16.0—	9.4+	42.7
20 Minus Ca	27.6	17.6—	9.6+	47.1
2 × √2 × S.E.	2.6	1.5	.6	
"F" Nutrients	2.26	141.98	41.82	
1% point	2.03	2.03	2.03	
"F" Replicates	1.50	2.77	3.04	
5% point	2.49	2.49	2.49	

+ and — indicate that these values are significantly higher or lower than those for the complete solution.

TABLE VII

EFFECT OF INCREASING THE IONIC CONCENTRATION OF PHOSPHORUS, POTASSIUM, NITROGEN, AND CALCIUM UPON THE NUMBER, HEIGHT, INFECTION RATING, AND WEIGHT OF MARQUIS WHEAT SEEDLINGS GROWN IN SILICA SAND, INFESTED WITH *Helminthosporium sativum*

Nutrient solutions	Number of plants	Height, cm.	Infection rating	Weight, mg.
1 H ₂ O (dist.)	27.6	18.3—	8.9+	23.2
2 Complete	28.0	32.1	4.6	72.1
3 300% P	27.4	30.8	4.8	59.1
4 500% P	28.8	31.8	4.1—	57.6
5 700% P	28.8	31.1	4.2	54.2
6 900% P	26.8	29.3—	4.1—	50.0
7 1100% P	27.6	26.1—	5.2+	41.3
8 133% K	27.6	31.5	4.8	87.7
9 167% K	27.6	31.3	4.2	85.5
10 200% K	28.2	31.3	4.3	85.1
11 233% K	28.6	29.9	4.4	71.3
12 267% K	25.4—	28.8—	4.1—	78.0
13 113% N	28.0	33.2	4.5	70.7
14 127% N	28.6	28.4—	4.6	57.5
15 140% N	28.2	29.9	4.0—	57.5
16 153% N	28.8	28.5—	4.3	55.6
17 167% N	25.4—	27.5—	4.5	55.9
18 140% Ca	28.0	30.8	4.5	82.9
19 180% Ca	26.8	30.7	4.3	83.6
20 220% Ca	26.6	28.8—	4.3	68.4
21 260% Ca	26.4	26.3—	4.6	59.1
22 300% Ca	26.0	24.3—	4.8	48.5
2 $\times \sqrt{2} \times$ S.E.	2.1	2.5	0.5	
"F" Nutrients	2.27	13.44	38.41	
1% point	2.03	2.03	2.03	
"F" Replicates	1.41	.57	.10	
5% point	2.49	2.49	2.49	

+ and — indicate that these values are significantly higher or lower than those for the complete solution.

phosphorus, potassium, nitrogen and calcium above the concentration of these ions in the complete solution did not appreciably reduce the development of the disease. However, it is recognized that the complete solution is not necessarily of such concentration as to give maximum growth. If the concentration had been increased slightly above that for maximum growth, it is possible there would have been an effect on disease development. Again, the F value for nutrients exceeded the 1% point for number, height, and infection rating.

Studies with Helminthosporium sativum in Pure Culture

The purpose of this study was to secure additional information concerning the effect of the various concentrations of phosphorus, potassium, nitrogen and calcium on the vegetative growth of *H. sativum*. All nutrient solutions used for the silica sand series were tested, the only difference being that a 2% dextrose solution was added to each formula. The tests were run simultaneously with those made in the silica sand (Expts. I, II, III and VIII).

The media thus prepared were distributed in 125-cc. quantities in 200-cc. Erlenmeyer flasks, and sterilized. Four flasks of each medium were inoculated with a 2-mm. loop of a spore suspension of the pathogen, and incubated at room temperature. At the end of 40 days, the mycelial mats were filtered off, dried at 90° C. for 48 hr., and weighed.

The results, which are given in Table VIII, indicate that the growth of *H. sativum* was seriously decreased only where there was either an omission or an excess of nitrogen, and also in the non-nutrient solution. This was particularly evident where there was an excess of nitrogen in the ammonium form, as in Solution No. 8 in Experiments I and II, and also in Solutions Nos. 13 to 17, inclusive, in Experiment VIII. Obviously nitrogen in the nitrate form was very important as a food element. Thus, of the four elements

TABLE VIII

EFFECT OF VARYING THE IONIC CONCENTRATION* OF PHOSPHORUS, POTASSIUM, NITROGEN, AND CALCIUM UPON THE DRY WEIGHT OF THE VEGETATIVE GROWTH OF *Helminthosporium sativum* IN PURE CULTURE

Omission and excess		Deficiency and excess		Decreasing amounts		Increasing amounts	
Nutrient solutions	Weight, mg.	Nutrient solutions	Weight, mg.	Nutrient solutions	Weight, mg.	Nutrient solutions	Weight, mg.
1	28.5	1	23.0	1	37.5	1	35.8
2	506.8	2	570.2	2	570.3	2	532.0
3	435.8	3	515.3	3	701.3	3	480.0
4	444.3	4	658.3	4	656.7	4	474.8
5	344.2	5	697.7	5	667.5	5	407.2
6	335.8	6	689.0	6	681.7	6	404.8
7	48.3	7	733.7	7	683.0	7	382.0
8	94.7	8	113.3	8	128.5	8	566.7
9	351.0	9	558.3	9	683.7	9	664.7
10	558.0	10	638.0	10	542.7	10	549.3
				11	450.0	11	645.3
				12	231.8	12	566.5
				13	167.0	13	218.0
				14	45.1	14	264.8
				15	396.2	15	240.7
				16	392.2	16	214.0
				17	506.8	17	260.0
				18	464.3	18	674.5
				19	481.2	19	657.5
				20	476.3	20	646.3
						21	652.7
						22	826.5

* The composition of the solutions in each series is identical with those used for Experiments I, II, III and VIII, respectively.

indicated, it would appear that in normal soil the availability of nitrogen in the nitrate form would really be the most important for the growth of the pathogen.

Discussion

In the work reported, an attempt was made to study, in pure silica sand culture, the incidence of foot rot (*H. sativum*) of the wheat plant in the seedling stage, as affected by four major elements, namely, phosphorus, potassium,

nitrogen and calcium, necessary to plant nutrition. The data do not necessarily apply to the mature stage of the host. Special attention was directed to the effect, on both plant and disease, of the absence, the deficiency, or the excess of minerals indicated.

The disease damage was interpreted according to such pathological manifestations as severity and extent of lesioning, stunting, and outright killing of the plants. Although this method is qualitative, and the one commonly used, quantitative measurements on height and weight of plants proved to be equally satisfactory.

One might naturally expect the wheat plant in an impoverished state, or in an unnatural environment, to be predisposed to attack by *H. sativum*. The data from these experiments strongly support this, since wherever a particular treatment, minus the pathogen, favored the production of undernourished plants, the disease was distinctly accentuated in the parallel series containing the pathogen. This, of course, indicates how closely the lack of host vigor and susceptibility to disease are related.

These studies show that the ions, K, N, and Ca, are necessary in the nutrition of the wheat plant during seedling stage in considerable amounts, if it is to escape excessive damage from the pathogen. On the other hand, the phosphorus ion does not so markedly influence the disease situation, in the seedling stage, for the limits of its effectiveness in maintaining the plant in a vigorous condition could not be measured under the condition of our experiments. Further, optimal concentrations of the K, N, and Ca ions clearly exist. These separate optima vary widely in magnitude. Two general observations seem applicable in the case of all three ions. Decreasing concentrations below the optimum predisposes the plant to more severe disease damage, while the moderate increases above the optimum do not materially affect the plant's response to the parasite. In field fertilizing practice it is possible that the same general principles would apply, although we have not yet attempted to verify this assumption.

Acknowledgments

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INFLUENCE OF AIR TEMPERATURE AND SOIL MOISTURE SUBSEQUENT TO FLOWERING ON THE NITROGEN CONTENT OF WHEAT¹

By J. W. HOPKINS²

Abstract

Marquis wheat plants were grown in soil in the greenhouse under uniform conditions until the flowering stage, when six differential treatments, viz., 15, 20 or 25% soil moisture in combination with a diurnal air temperature cycle of 45–70° or 55–80° F., were imposed.

The effect of the higher air temperature in increasing nitrogen content through accelerated respiration was evident in grain collected when in the early dough stage (about 50% dry matter). By the late dough stage there were also significant differences attributable to soil moisture under both temperature regimes. However, the nitrogen content of the completely ripe grain was practically the same for all six treatments. This is attributed to a retardation of maturity by both increased soil moisture and lower air temperature, which would permit additional dissipation of carbohydrate through prolonged respiration, and also possibly to differences in the extent of tillering.

Compensatory effects of this magnitude would hardly be expected under field conditions, but might occur on a reduced scale, thus increasing the difficulty of correlating nitrogen content with meteorological observations.

In a previously reported (4, 5) statistical study of the influence of weather conditions on the nitrogen content of wheat grown in experimental plots at various stations in western Canada, it was found that above-average precipitation in the earlier part of the growing season was associated with below-average nitrogen content, but that the data did not demonstrate any correlation between nitrogen content and rainfall during the later stages of development. On the other hand air temperature during this latter period was positively correlated with nitrogen content when weighted on the supposition that the rate of respiration was doubled by each 10° C. rise in temperature.

The correlations indicated were, however, only moderate, partly no doubt because of variations introduced by the fact that the plots were not permanent, but were grown on different soil at each station each year. There was thus an appreciable possibility of actually significant effects escaping detection, and it seemed desirable to obtain further data under more closely controlled conditions. The greenhouse experiment described below was therefore undertaken with this end in view.

Experimental

Briefly, the experiment consisted in growing 540 Marquis wheat plants under uniform conditions until the flowering of the main tillers. At this juncture two different levels of air temperature and three levels of soil moisture, making six treatment combinations in all, were imposed upon randomly selected groups of 90 plants and maintained until the conclusion of the experi-

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ment. Collections of the resulting grain, which were made at each of three stages of maturity, were weighed and analyzed for dry matter and nitrogen content.

The crop was grown in 180 numbered 1-gal. glazed earthenware crocks, the tare weights of which were equalized by the addition of varying amounts of coarse (No. 6) quartz sand. Steam-sterilized loam soil (5 kg.) was firmed into each crock, and six grains of Marquis wheat (kindly supplied by the Cereal Division, Central Experimental Farm, Ottawa) planted at a depth of one inch below the surface on October 20, 1936. On November 12 the number of seedlings per crock was reduced to three.

The 180 crocks were arranged in 10 rows of 18 on two benches in a unit of the greenhouse having diurnal temperature control. Each row provided one crock for each of the 18 combinations of treatment and date of harvesting. Since the object was to obtain as accurate as possible a comparison between the six moisture and temperature treatments at each date of harvesting, the 18 crocks in each row were first divided into three groups of six, and one group chosen by random selection for each date of harvesting. Then the six crocks within each such group were allotted at random to the six combinations of temperature and moisture to be subsequently imposed. There were thus ten parallel blocks or groups of crocks, each comprising the six treatments, available for harvesting at each of the three dates.

When weighed into the crocks, the soil contained approximately 20% moisture (oven-dry basis). After seeding, this was brought up to 25% by placing each crock in turn on the scales and adding water until the crock and contents attained the pre-calculated total weight corresponding to that moisture content. By means of regular weekly weighings, the moisture supply of all crocks was then maintained at this level until the date of flowering.

In order to promote uniformity within parallels, the location of the individual crocks within each set of six underwent a cyclical permutation, one move in the permutation being made on the occasion of each weighing and watering. By the repetition of this process positional effects in lighting, temperature and air currents within groups were to some extent equalized.

As the plants increased in size, weekly watering became inadequate, and was supplemented by a mid-weekly supply which was distributed uniformly to all crocks in an amount dependent on the rate of utilization, as indicated by weighing. Actual weighing and movement of the crocks was, however, restricted to weekly intervals.

An initial diurnal temperature cycle of 35-55° F. was adopted, which was progressively raised to 45-70° F. at the time of flowering. When the flowering stage had been passed by the main tillers and attained by the second and third tillers, the 90 crocks allotted to the higher temperature regime were transferred to the adjacent greenhouse unit. The two units now employed were similar in all respects except that diurnal temperature cycles of 55-80° and 45-70° F. were maintained. At the same time three levels of soil moisture

were imposed, the water supply being replenished to 15, 20 and 25% of the oven-dry soil at each weekly weighing, and corresponding modifications being made in the amounts supplied mid-weekly. The principle of arrangement of the crocks on the benches remained the same, each original group of six now being represented by a sub-group of three in each unit. The weekly permutation of crock positions within sub-groups continued as before.

Collections of heads were made at three stages of maturity, *viz.*, early dough, late dough and dead ripe. The first collection was made simultaneously in both units, but as the higher temperature regime perceptibly accelerated senescence, the second was separated by an interval of 12 days in order to secure kernels at approximately the same stage of development. There was a difference of nearly three weeks in the date of attainment of dead ripeness.

It was impossible to thresh out the kernels obtained in the first two collections, which accordingly had to be removed from the heads individually by hand. This was done as expeditiously as possible. After counting and weighing, the kernels from each crock were dried *in vacuo* at 98° C. for approximately 15 hr. in order to destroy enzyme activity which, by the dissipation of carbohydrates, might have led to fictitiously high nitrogen values (6). The dried kernels were subsequently exposed to the atmosphere of the laboratory for three or four days, again weighed, and ground in a micro-mill of the shearing type. Duplicate one-gram samples of the ground material were taken immediately after grinding for the determination of the moisture content of the air-dry grain, which was effected by further drying for one hour in an air oven at 130° C. Finally, the nitrogen content of these samples was determined by the Kjeldahl method, selenium being employed as a catalyst.

Vegetative Features

Results

Table I shows the average number of culms, heads and grain-bearing heads per plant in the 10 replicate crocks of each treatment combination, harvested at the three stages of development indicated above. Under the conditions of

TABLE I
AVERAGE NUMBER OF CULMS AND HEADS PER PLANT AT HARVEST

Collection	Soil moisture	Culms		Heads		Grain-bearing heads	
		45-70° F.	55-80° F.	45-70° F.	55-80° F.	45-70° F.	55-80° F.
First	15%	4.4	4.3	4.1	3.6	3.2	3.3
	20%	7.4	4.2	4.4	4.2	3.1	4.0
	25%	9.0	4.7	4.5	4.5	3.3	3.5
Second	15%	5.1	4.2	4.7	3.8	3.3	3.7
	20%	8.2	4.1	7.3	3.8	3.5	3.2
	25%	10.5	5.1	9.8	4.4	3.7	3.9
Third	15%	5.9	3.9	5.4	3.7	3.8	3.5
	20%	9.0	5.0	8.9	4.4	6.5	3.8
	25%	10.6	5.0	10.4	4.9	8.1	4.1

lower temperature (45–70° F.), there was a considerable proliferation of late tillers at the two higher soil moistures. At the time of the first harvesting, few or none of these had produced heads. By the second harvesting, however, most had headed out, although they were as yet devoid of kernels, and at maturity an appreciable number yielded small quantities of grain. At the higher temperature, on the other hand, this tendency was reduced to very minor proportions.

TABLE II
AVERAGE NUMBER OF KERNELS PER CROCK, FRESH WEIGHT, AND WEIGHT PER THOUSAND

Collection	Soil moisture	Number of kernels		Fresh wt., gm.		Fresh wt. per 1000 kernels, gm.	
		45–70° F.	55–80° F.	45–70° F.	55–80° F.	45–70° F.	55–80° F.
First	15%	267	253	15.2	11.6	56.6	45.1
	20%	276	268	15.6	13.5	55.8	50.5
	25%	274	281	15.4	15.0	56.1	53.3
Second	15%	272	279	14.8	11.6	54.2	41.9
	20%	279	275	15.8	13.0	56.6	47.6
	25%	294	290	16.9	14.1	57.4	49.0
Third	15%	277	277	11.7	9.0	42.3	32.6
	20%	352	288	13.2	9.9	38.0	34.6
	25%	439	296	16.2	10.2	37.4	34.6

The greater vegetative growth under the lower temperature regime is reflected in the average number of kernels per crock of the mature plants, and in the fresh weight of grain secured from both of the last two collections, as shown in Table II. The fresh weight per 1000 kernels of the mature grain suggests that the later tillers produced a preponderance of small or shrivelled kernels, but even so the average is higher than that for the 55–80° temperature regime. Although the latter apparently inhibited late tillering, there was nevertheless a consistent increase in both the fresh weight and weight per 1000 kernels from the 15 to the 25% moisture level at all three collections.

First Collection

When the first collection of heads was taken, the average dry-matter content of the grain from the 30 crocks harvested in the lower temperature unit was 43.4%, whereas the corresponding figure for the higher temperature was 55.7%. Consequently, although the lower temperature gave the greater fresh weight of grain, as indicated in Table III, the actual yield of dry matter at this time was on the average slightly higher in the 55–80° unit, namely 7.4 as against 6.7 gm. per crock. The difference, however, is insignificant.

The variance of the individual yields of dry matter per crock within each unit was analyzed by the well known procedure of Fisher (3) into components ascribable to differences between parallel groups, differences between soil moistures, and residual variation or error. As expected, there were marked positional effects in both units, which gave rise to highly significant mean

TABLE III
AVERAGE DRY-MATTER CONTENT, YIELD AND NITROGEN CONTENT OF GRAIN, FIRST COLLECTION
(10 REPLICATES)

	Diurnal temp. 45-70° F.				Diurnal temp. 55-80° F.			
	Soil moisture			Average	Soil moisture			Average
	15%	20%	25%		15%	20%	25%	
Average dry-matter content, %	44.0	43.3	42.9	43.4	58.5	54.5	54.1	55.7
Average yield of dry matter, gm. per crock	6.7	6.8	6.6	6.7	6.7	7.3	8.1	7.4
Average nitrogen content, % (dry basis)	3.49	3.45	3.41	3.45	3.75	3.78	3.74	3.76

squares between parallels in both analyses of variance. The layout of the experiment was such that average differences between parallels are eliminated from treatment comparisons, which are made within parallels. For the 45-70° temperature regime, the differences in mean yield between the three levels of soil moisture subsequent to flowering shown in Table III are totally insignificant. At the higher temperature, however, there is a significant increase in the yield of dry matter with each increase in soil moisture. The residual mean square error was essentially the same in both cases.

Similar analyses of the variance of the nitrogen content of the grain secured from each crock revealed no significant differences between parallels or between soil moistures at this stage for either temperature. There is, however, as indicated in Table III, an average difference in nitrogen content of 0.31% in favor of the higher temperature regime, which significantly exceeds its standard error of 0.096%. The average nitrogen content, namely 3.45 and 3.76%, is in both cases quite high, corresponding to a protein content on the conventional 13.5% moisture basis of 17.0 and 18.5% respectively.

Second Collection

It has already been mentioned that an interval of 12 days separated the second collection of heads from the higher and lower temperature units. In this way, material at approximately the same stage of maturity was secured, as indicated by the average dry-matter contents of 66.8 and 63.5% respectively shown in Table IV. It is to be observed from this table however that in addition to the effect of temperature in accelerating maturity (as indicated by desiccation) there was also by this time a similar but less pronounced effect within each temperature regime, attributable to the imposed differences in soil moisture supply.

Table IV also shows the yield and nitrogen content of the grain secured from this collection. The yield of dry matter is now somewhat higher than that obtained in the previous collection, particularly in the case of the 45-70° temperature regime, for which the average is 10.1 gm. per crock, as compared with 8.6 gm. for the 55-80° diurnal sequence. The difference,

TABLE IV
AVERAGE DRY-MATTER CONTENT, YIELD AND NITROGEN CONTENT OF GRAIN, SECOND COLLECTION
(10 REPLICATES)

	Diurnal temp. 45-70° F.				Diurnal temp. 55-80° F.			
	Soil moisture			Average	Soil moisture			Average
	15%	20%	25%		15%	20%	25%	
Average dry matter content, %	65.4	63.0	62.0	63.5	69.5	66.2	64.8	66.8
Average yield of dry-matter, gm. per crock	9.7	10.0	10.5	10.1	7.8	8.6	9.1	8.6
Average nitrogen content, % (dry basis)	3.77	3.68	3.51	3.65	3.92	3.74	3.79	3.82

1.5 \pm 0.37 gm., is statistically quite significant. As before, an analysis of variance showed no significant effect of soil moisture subsequent to flowering upon yield under the lower temperature regime, the late tillers, although well headed out, being as yet devoid of grain. At the higher temperature there is again a consistent increase in mean yield for each increase in soil moisture which, taken in conjunction with the previous results, is suggestive of a real effect, even though the analysis of variance gave in this instance a mean square falling somewhat below the 5% level of significance. The mean square error in the two cases was again of the same order.

At this juncture, the average nitrogen content of the grain developing under the 45-70° temperature cycle was 3.65%, 0.20% higher than that for the first collection, whilst that for the 55-80° temperature, 3.82%, did not differ significantly from the earlier value of 3.76%. The difference between the averages for the two temperature regimes, namely 0.17 \pm 0.05% in favor of the higher temperature, is again significant. In addition, there is now a demonstrable effect of soil moisture under both temperature conditions, higher soil moisture being associated with lower nitrogen, except in the case of 25% soil moisture at 55-80° F., which does not differ significantly from the 20% series.

The possibility of correlation between grain yield and nitrogen content was investigated by partitioning the total covariance of these quantities within each temperature regime in the manner already employed in the analyses of variance, *i.e.*, between parallel groups, between soil moistures, and residual. In both cases the residual covariance gave rise to a small negative correlation coefficient, but neither of these attained the 5% level of significance.

Final Collection

The plants under the higher temperature cycle matured more rapidly, and hence were actually harvested prior to those in the adjoining unit. As indicated in Table V, the average dry-matter content of the grain at the time of collection, namely 90.3 and 89.4%, was much the same in both cases.

Table V also shows the average yield of grain, expressed as grams of dry matter per crock, and the average nitrogen content of each series. The average yield for the 10 replicate crocks of the 45-70° series, 12.3 gm., is significantly higher than the corresponding figure of 8.9 gm. for the 55-80° series. There is now a significant effect of soil moisture upon yield in both series, but this is much more pronounced at the lower temperature owing to the fact that many of the late tillers, when allowed to mature, produced small quantities of grain. Positional effects, reflected in the differences between the average yields of the 10 replicate groups of each series, were again highly significant in the analyses of variance, as in the two previous collections. The residual mean square error was somewhat higher in the 45-70° than in the 55-80° series, the yield of grain from the additional tillers of the former doubtless introducing an extra source of variability.

TABLE V
AVERAGE DRY-MATTER CONTENT, YIELD AND NITROGEN CONTENT OF GRAIN, FINAL COLLECTION
(10 REPLICATES)

	Diurnal temp. 45-70° F.				Diurnal temp. 55-80° F.			
	Soil moisture			Average	Soil moisture			Average
	15%	20%	25%		15%	20%	25%	
Average dry-matter content, %	89.7	89.3	89.1	89.4	90.4	90.3	90.3	90.3
Average yield of dry matter, gm. per crock	10.5	11.8	14.5	12.3	8.2	9.0	9.3	8.8
Average nitrogen content, % (dry basis)	3.83	3.94	3.81	3.86	3.84	3.83	3.92	3.86

Turning now to the nitrogen content of the ripe grain, given in the bottom line of Table V, it will be observed that the average for both temperature regimes is now the same at 3.86%. Furthermore, analyses of variance indicated that the small differences between the averages for the three soil moisture levels were in both cases insignificant, although the pooled residual covariance of yield and nitrogen content gave rise to a small but probably significant correlation coefficient of -0.38, and, as noted in the preceding paragraph, there are undoubted differences in yield attributable to both moisture and temperature conditions. This final uniformity of nitrogen content is believed to be due to the fortuitous balancing of opposed effects, since definite differences were discernible in the developing grain.

In this connection, three points may be noted. (i) The grain under the 55-80° temperature regime was fully ripe on the average between two and three weeks earlier than that under the 45-70° regime. (ii) Soil moisture was maintained at the predetermined level of 15, 20 or 25% right up to the time of the final harvesting. This resulted in differences in date of maturity at both temperatures, the plants receiving the smaller quantities of moisture maturing more rapidly. It may be assumed from the results of the second

collection of immature kernels, summarized in Table IV, that the additional vegetative growth resulting from increased soil moisture led to some dilution of the nitrogen content of the parent material translocated to the grain. On the other hand, however, the additional moisture supply operated to retard desiccation, thus permitting a prolongation of active respiration, which in the end apparently sufficed to offset the former effect. Similarly, the results in both Tables III and IV indicate that the higher diurnal temperature cycle caused an increased dissipation of carbohydrate through respiration. Here again, however, desiccation supervened earlier, and the lower rate but more prolonged duration of respiration at 45-70° apparently contributed to an equivalent result. (iii) The final production of grain by a number of the late tillers generated under this temperature regime may also have operated in the same direction, since Engledow and Wadham (2) found the percentage of nitrogen in the grain of mature ears of barley to increase in sequence from the main axis down through the successive side tillers.

Since the experimental conditions were in certain respects artificial, the foregoing compensatory effects are doubtless exaggerated in comparison with those occurring under field conditions in western Canada. Apart from the tillering differences noted, the maintenance of a continuous supply of soil moisture during maturation is a situation hardly likely to be encountered in practice. Also, it has long been known (1, p. 68) that the respiration of greenhouse plants is abnormally high. However, the presence of such effects, even in moderate degree, may contribute to the difficulty encountered in correlating nitrogen content with the meteorological conditions prevailing during the formation and ripening of grain crops.

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NOTE ON SULPHANILAMIDE AND OTHER CHEMICALS THAT ACT AS PLANT GROWTH PROMOTING SUBSTANCES¹

BY N. H. GRACE²

The response of both higher and lower plant forms to growth stimulating chemicals suggested a possible relationship between the action of the so-called phytohormones and that of other chemicals or drugs used in controlling bacterial growth. A study of several plant extracts indicated the presence of active material not to be accounted for by heteroauxin or auxin as such. The chemical separation of the active materials was started by Dr. W. E. Graham of these laboratories: his work was cut short by his untimely death, and in consequence, a detailed report on the project has been delayed. However, his work suggested testing a number of chemicals for their physiological activity as growth promoting substances, including some of natural plant origin and others which are products of artificial synthesis, such as sulphanilamide.

The yeast method of testing was used as an indicator of activity (3). Following this, the chemicals were applied to cuttings of easily rooted plants. The response was compared with that obtained with indolyl-3-acetic acid. The physiological curve, already reported as characteristic of the effects of hormones, was demonstrated for these additional substances by application to tomatoes grown in sand culture. In some cases a response was noted with seeds.

Another method of determining the activity of the recognized plant hormones and new chemicals is being tested independently by Dr. N. E. Gibbons and Dr. G. A. Ledingham of these laboratories, who will report their experiments in due course. They verified inhibitory concentrations of substances mentioned herein by the clear zone of inhibition occurring around a pellet of talc containing the active chemical, applied to the surface of Petri dish cultures of bacteria or fungi.

The foregoing methods indicated that coumarin, vanillic acid, 1- and 2- γ -naphthyl butyric acid (3) and sulphanilamide had a definite measure of activity as growth promoting substances. The response was comparable to that shown by both indolyl-3-acetic and 1-naphthyl acetic acids, though it was not as great in all cases. In lesser measure, piperonal, methoxy-salicylaldehyde and vanillin also indicate activity. Observations with yeast alone indicate some activity for iso-vanillin, piperonal acetic acid and colchicine.

Perhaps the most interesting results are those obtained with sulphanilamide. It gives a response with yeast and stimulates the proliferation of roots by certain plants that root readily. A physiological curve of stimulation and inhibition follows its application to tomatoes in sand culture. A clear-cut

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response follows its addition to seeds. In all these cases sulphanilamide acts like the recognized plant hormone chemicals. It would appear to follow that sulphanilamide does possess some growth promoting properties.

This similarity in the behavior of plant hormones and sulphanilamide suggests that the therapeutic effectiveness of the latter may be attributed, in some measure at least, to its hormone-like properties. In other words, the effect on bacterial infections may be due to overdosage. The concentrations employed therapeutically are such that inhibition of growth results; inhibition similar in nature to that shown by higher plants, fungi or bacteria, when they are treated with excessive doses of indolyl-3-acetic or 1-naphthyl acetic acids.

Reference to the current literature on sulphanilamide and the group of related chemicals indicates no clear concept of the mechanisms involved (1, 4). The analogy with phytohormones would suggest looking for a common cause and mode of action. Two conclusions would appear to follow. In the first place, certain low concentrations of sulphanilamide might be expected to stimulate, rather than control, the infection. With phytohormones it is usually much easier to show inhibition than stimulation. Moreover, a number of factors are involved in stimulation and it may be difficult to show it under the complex conditions which exist for growth of organisms in the animal body. In the second place, phytohormones should be considered as a possible means of checking the growth of undesirable micro-organisms.

In conclusion, it may be pointed out that other apparently active chemicals are under study in this laboratory. Various workers report numerous materials affecting plant growth (2). Where may the line be drawn between a true phytohormone and a material exhibiting toxic effects at high concentrations and stimulating effects at low? The number of active materials now known suggests some such division is desirable.

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ANALYSIS OF FLUCTUATIONS IN THE ACTIVITY OF INSECTS. A STUDY ON THE EUROPEAN CORN BORER, *PYRAUSTA NUBILALIS* HUBN.¹

BY GEOFFREY BEALL²

Abstract

The numbers of moths in flight, from night to night, over areas on which observations were made concurrently, show high correlations, although catch on non-contiguous areas differed more than would have been expected by chance. Polynomials of the fourth degree were found the most satisfactory relations to fit in determining seasonal trend of flight for each year.

The relations between numbers of moths in flight and physical conditions in the evening were investigated by the variate-difference procedure, using first differences. The numbers of moths and evening temperature showed a consistent moderate positive correlation. The numbers of moths and daily maximum temperature were positively correlated but the partial correlation, holding evening temperature constant, showed no relationship. The numbers of moths and evening humidity were not correlated, either simply, or as a partial correlation holding evening temperature constant. The numbers of moths and evening wind velocity, both simply and as a partial correlation holding evening temperature constant, showed a consistent moderate negative correlation.

For the relation between temperature and activity two successive approximations to a polynomial of the third degree were fitted jointly to all years. For temperatures below 60° F. there was no appreciable activity on the part of the moths. Activity increased with rise in temperature, although less rapidly as temperature became high. In general, activity at a given temperature was greater as the temperature of the preceding day was low.

General Problem

It is generally accepted that environmental conditions affect the activity of insects. Many observations on the activity and rate of development of insects, under controlled physical conditions, show these phenomena to vary particularly with temperature.

In order to determine the nature of the relations between activity and physical conditions, entomologists have recorded activity in the field and have made observations on meteorological conditions, concurrently. In such work, it is desired to know what activity would have occurred at any given time under conditions other than those observed, or in places other than those where observations had been made. It is desired to evaluate times and localities with respect to their suitability for insect activity. Such a study has been made for some years at the Chatham laboratory on the European corn borer, *Pyrausta nubilalis* Hubn.

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In this paper the relation between activity of *Pyrausta nubilalis* Hubn. and physical conditions is examined. The methods of examination may be of use for insects in general. The sampling error of such types of observations is considered. The best means for estimating activity from a knowledge of physical conditions is sought.

Historical Summary of Work on Problem

A short summary of efforts to find the relation between activity and physical conditions will give an idea of the standing of the problem. It may be noted that problems on rate of development are generally considered very similar to problems on degree of activity. In much of this work some preconceived relationship is fitted to data by subjective methods.

McEwan and Michael (7) use a method of solving for the relation between rate of biological processes and meteorological conditions. These workers break the series of values of one meteorological condition into groups, and then find the average value of the biological process rate for each group to obtain a first approximation to a relationship. The effect of the first meteorological condition is eliminated on the basis of this relationship and a first approximation to the partial relationship for the second obtained. Successive approximations back and forth between the two conditions are made. Cook (4) studies the relation between numbers of moths flying into bait traps and temperature. He correlates deviations from a seasonal trend with temperature, but determines no regression. The seasonal trend is found by the use of a sliding average. Glenn (5), in a study on rate of codling moth development, assumes a Λ -shaped relationship, between rate of development and temperature, to exist. He tries various values for the feet and peak until he gets what he considers to be the best relationship. In studies on development under controlled physical conditions, Shelford (9) fits, by eye, a Krogh curve for rate of development against temperature. Williams and Bishara (15), in a study on activity of butterflies, take the average flight for each day of a particular temperature as the number of insects to be expected at that temperature. A comparison between the numbers at different temperatures gives the relative activity for the two temperatures. Janisch (6) works on the relation of activity to temperature and upon the effect of a given temperature upon subsequent activity. He fits curves upon the basis of the temperature at which activity is maximal.

Data Employed

Data on the numbers of moths of *Pyrausta nubilalis* Hubn., taken at Chatham, Ontario, are considered in this paper. The number given for each evening is that observed in a plot of field corn during the years 1927 to 1933, inclusive. These observations and also meteorological readings were made at intervals of an hour during each evening. In 1927, moth flight was recorded in four sub-plots, and in 1929, flight was recorded in a field adjacent to the laboratory plot. All these data are presented in Table I. It may be noted

that in 1927 the collections made before July 11 are not comparable to those made later in the season and that the values for 1928 were doubled in a previous publication (13) for reasons stated therein.

TABLE I
NUMBER OF MOTHS OBSERVED IN FLIGHT DURING ENTIRE EVENING

Date	1927					1928	1929		1930	1931	1932	1933
	Sub-plot 1	Sub-plot 2	Sub-plot 3	Sub-plot 4	Total		Laboratory plot	Secondary plot				
June 22												—
23												0
24												10
25												3
26									—			15
27									0		—	30
28									0	—	3	6
29									1	0	8	36
30									14	1	1	33
July 1							•		0	1	0	14
2							—	—	0	1	0	0
3							0	0	0	0	0	14
4							1	0	8	6	0	22
5							4	4	10	4	11	28
6						—	0	2	3	3	16	29
7	—	—	—	—	—	1	14	22	5	2	1	22
8	1	1	0	0	2	0	8	21	55	5	21	9
9	0	2	0	2	4	6	6	24	24	9	16	7
10	14	3	5	4	26	0	0	1	25	0	20	4
11	4	3	5	12	24	5	1	7	3	2	12	3
12	8	9	26	30	73	5	12	24	4	7	19	12
13	18	25	17	56	116	0	3	24	1	12	8	6
14	11	16	29	41	97	9	1	3	0	4	9	8
15	12	17	12	12	53	4	4	12	1	6	2	4
16	15	23	20	34	92	32	5	10	6	3	9	2
17	9	15	13	13	50	12	14	43	7	0	7	1
18	18	2	7	26	53	18	0	0	0	3	5	3
19	0	0	0	0	0	12	0	0	0	3	6	2
20	2	3	5	6	16	12	0	1	1	0	2	1
21	8	25	23	34	90	14	3	6	0	0	0	0
22	2	0	2	11	15	17	6	12	0	0	3	1
23	2	3	9	16	30	4	6	11	0	0	0	0
24	2	3	3	13	21	8	2	2	1	0	2	0
25	5	3	8	7	23	3	3	6	0	0	0	0
26	2	3	3	11	19	2	3	2	0	0	0	—
27	1	4	5	11	21	7	4	3	0	0	0	
28	1	2	2	5	10	0	2	6	—	0	0	
29	0	0	0	0	0	0	3	1		0	0	
30	1	5	2	8	16	2	2	1		0	—	
31	1	0	1	3	5	3	1	0				
Aug. 1	0	0	0	0	0	0	0	0		—		
2	0	0	0	0	0	3	0					
3	0	0	0	3	3	3	0	—				
4	0	0	0	0	0	0	0					
5	0	0	0	2	2	0	0					
6	0	2	1	1	4	0	0					
7	1	1	0	0	2	1	0					
8	0	0	0	0	0	0	0					
9	—	—	—	—	—	2	—					
10						0						
11						—						

TABLE II
PHYSICAL CONDITIONS AT THE TIME OF FLIGHT OF MOTHS

Date	1928			1929			1930			1931			1932			1933				
	Even. temp.	Max. temp.	Humid.	Wind vel.	Even. temp.	Max. temp.	Humid.	Wind vel.	Even. temp.	Max. temp.	Humid.	Wind vel.	Even. temp.	Max. temp.	Humid.	Wind vel.	Even. temp.	Max. temp.	Humid.	Wind vel.
June 22																				
23																	70	79	76	24
24																	72	82	60	
25																	75	85	76	63
26																	79	87	73	4
27																	82	—	—	34
28																	76	99	68	8
29																	82	95	73	5
30																	57	81	87	73
July 1																	70	85	67	73
2																	68	78	70	22
3																	64	80	67	49
4																	63	76	60	82
5																	59	60	65	28
6																	63	72	74	4
7																	58	67	67	60
8																	71	82	71	85
9																	72	82	86	5
10																	67	75	75	11
11																	68	81	81	63
12																	71	85	73	85
13																	71	82	86	6
14																	67	82	61	17
15																	67	80	81	56
16																	67	82	61	14
17																	67	81	68	4
18																	67	80	64	14
19																	67	82	63	29
20																	67	85	77	12
21																	67	85	69	85
22																	67	85	69	85
23																	67	85	69	85
24																	67	85	69	85
25																	67	85	69	85
26																	67	85	69	85
27																	67	85	69	85
28																	67	85	69	85
29																	67	85	69	85
30																	67	85	69	85
31																	67	85	69	85
1																	67	85	69	85
2																	67	85	69	85
3																	67	85	69	85
4																	67	85	69	85
5																	67	85	69	85
6																	67	85	69	85
7																	67	85	69	85
8																	67	85	69	85
9																	67	85	69	85
10																	67	85	69	85
11																	67	85	69	85
12																	67	85	69	85
13																	67	85	69	85
14																	67	85	69	85
15																	67	85	69	85
16																	67	85	69	85
17																	67	85	69	85
18																	67	85	69	85
19																	67	85	69	85
20																	67	85	69	85
21																	67	85	69	85
22																	67	85	69	85
23																	67	85	69	85

TABLE II—*Concluded*

PHYSICAL CONDITIONS AT THE TIME OF FLIGHT OF MOTHS—*Concluded*

[illegible]

The physical conditions half an hour after sunset are considered typical of an evening because the main flight of corn borers occurs shortly after sunset. In Table II, evening temperature, in degrees Fahrenheit, evening relative humidity, as a percentage, evening wind velocity, in units of ten feet per minute, and also daily maximum temperature, in degrees Fahrenheit, are shown. General discussion on the method of observation has been published (12, 13).

Notes on the Life History of the European Corn Borer

Pyrausta nubilalis Hubn. hibernates as a mature larva and in the late spring goes into the pupal stage. Spencer (10), working on females of the Ontario race of corn borers in 1921 and 1922, found longevity of moths to be between 15 and 17 days and to range from 7 to 31 days. In this material the pre-oviposition period averaged slightly less than 4 days and ranged from 2 to 9 days. Working on the same race, Poos (8) found the duration of each stage to be about as long and even more variable. The latter worker also found emergence from the pupal stage to continue all through July. It can be seen that among corn borers where all members of a group were held under the same conditions, by these workers, there was great variability in the duration of the stages. Caffrey (2) found that moths may fly five miles; therefore dispersion must tend to equalize local fluctuations in the rate of development and in moth population, over a wide area, and must further spread the occurrence of corn borer moths in a given field.

Chance Error of Observations

It would be of use to see what magnitude of variation can be expected by chance in observations of n_s , the total number of moths seen in flight on the s^{th} evening. Let the s^{th} evening be any evening during the periods for which data were collected. Difficulty is encountered in estimating the magnitude of chance variations in observations of n_s because the observation of each evening is an isolated value and it is impossible, strictly, to repeat it. However, parallel observations were made in 1927 on four sub-plots and in 1929 on two plots. An examination is first made to determine to what extent catches made at one time on different areas were alike. Next, an examination is made to ascertain whether the catches on various sub-plots can be supposed to differ as they did, by chance. Finally, the results from this work are examined to determine in what way the magnitude of chance variation is related to the observed numbers.

To determine the similarity of the catch on the various sub-plots, the correlation between plots, for the numbers of moths taken each evening, is calculated. The data used run from July 8 to August 7, inclusive. The correlation coefficients between the data from the various pairs of sub-plots are as shown in Table III.

The correlation between catches from adjacent plots from which collections were made at one time is further investigated in the case of the two plots

TABLE III
CORRELATION BETWEEN CATCHES FROM DIFFERENT
SUB-PLOTS OF 1927

—	Sub-plot II	Sub-plot III	Sub-plot IV
Sub-plot I	+ .72	+ .70	+ .66
Sub-plot II		+ .84	+ .83
Sub-plot III			+ .86

The correlation between catches on the sub-plots of 1927 and on the two plots of 1929 is high. This result shows that the numbers of moths caught on any one area vary with the numbers caught concurrently on other areas. Therefore, the nightly fluctuations in the number of moths from the various areas were not peculiar to the individual areas. Apparently, whatever controlled flight over one area did the same over the other areas.

While the catch from the various sub-plots of 1927 and from the two plots of 1929 may be highly correlated, the correlation may be largely due to seasonal trend. In examining the data from the four sub-plots in 1927, the question at once arises of how far the number of moths in each agrees with that in the others, because the numbers fluctuate from night to night about the seasonal trend. It is obvious that the number of moths might increase or decrease in all the sub-plots at about the same time of year, yet the nightly deviations from this trend might be largely independent. Accordingly, parallel observations between the four sub-plots of 1927 are examined to see with what degree of probability the sub-plots can be considered parts of one sample. The method of attack is to suppose the flight in the four sub-plots to be essentially identical and then to consider the probability that the catch would have differed, by chance, as much as was observed. The four sub-plots are considered in pairs to find how far flight on each pair might be considered identical. The value of χ^2 for each pair of sub-plots is calculated.

The data are grouped for the evenings July 8 to 10, inclusive, for July 18 and 19, for July 20 and 21, for July 22 and 23, for July 24 and 25, and for the evenings July 26 to August 7, inclusive. Thus, for each sub-plot, thirteen values of reasonable magnitude are available. The results are as shown in Table IV.

Since with twelve degrees of freedom the 0.05 and 0.01 levels of probability for χ^2 are 24.054 and 26.217, respectively, it is apparent that only in two cases are non-significant values of χ^2 found. It is ap-

of 1929. The work resembled that done on the data from the sub-plots of 1927. The correlation between the catches from the laboratory plot and a secondary adjacent plot, in 1929, is calculated. The data used run from July 4 to July 31, inclusive. The correlation coefficient is +.85.

TABLE IV
VALUES OF χ^2 FOR CATCH FROM
VARIOUS SUB-PLOTS OF 1927

—	χ^2
Sub-plots I, II	32.915
Sub-plots I, II'	34.450
Sub-plots I, IV	44.327
Sub-plots II, III	22.451
Sub-plots II, IV	40.593
Sub-plots III, IV	22.050

parent that the catch from the pairs of contiguous plots, II and III, and III and IV, only, differed as little as can be expected by chance. The most widely removed plots, I and IV, differed to the greatest extent. Apparently there was some systematic variation in catch over the plot as a whole.

In order to see how the numbers of moths coming to a given plot should vary by chance, suppose one had had an infinite number of like plots to which moths might fly randomly. On the s^{th} evening a moth might go to any one of a large number of plots similar to that being used for observations. The probability of it coming to the laboratory plot would have been, therefore, very small. Since, however, many moths were available in a district, the number actually coming to any plot would have been moderately great. If the total number of moths coming to the given plot on the s^{th} evening were n_s , the mean number coming to all the plots would have been \bar{n}_s . The values of n_s would have varied about \bar{n}_s in a Poisson distribution.

As a study of whether the magnitude of the deviation of n_s really fell anywhere near $\sqrt{\bar{n}_s}$, the catches on the sub-plots of 1927 are supposed to be all part of one flight and the deviations of the various sub-plots are supposed to be due to chance. The catches have been found, above, to fluctuate together, although the discrepancies were a little greater than might be expected by chance. It is supposed that from the four sub-plots one can get for any one sub-plot an expectation of flight. This expectation is found in the same way that expectation is calculated in making the χ^2 test. Let n_{si} be the catch on the s^{th} night in the i^{th} plot, and ϵ_{si} be the corresponding expectation. Deviation of n_{si} from ϵ_{si} should vary with, approximately, $\sqrt{\epsilon_{si}}$ as a standard deviation. Accordingly, the values of $\frac{n_{si} - \epsilon_{si}}{\sqrt{\epsilon_{si}}}$ should be approximately distributed with standard deviation of unity.

Such values $\frac{n_{si} - \epsilon_{si}}{\sqrt{\epsilon_{si}}}$ are calculated for the data of the sub-plots of 1927. These values, called X , are plotted in Fig. 1 against the total number of moths, called Y , in the four sub-plots on the s^{th} evening. The data used run from July 8 to August 11, inclusive. In order to secure entries of satisfactory magnitude, observations are grouped for the evenings July 8 to 10, inclusive, for July 18 and 19, for July 28 to 30, inclusive, and for July 31 to August 11, inclusive. The ratio $\frac{n_{si} - \epsilon_{si}}{\sqrt{\epsilon_{si}}}$ is independent of meteorological conditions, or of seasonal trend on the various nights, since it is only concerned with the fluctuations on a single night.

In Fig. 1, it can be seen that of the 76 values of $\frac{n_{si} - \epsilon_{si}}{\sqrt{\epsilon_{si}}}$, 7 fall beyond ± 2 , 22 fall beyond ± 1 , and 47 fall within ± 1 . The corresponding figures from the normal curve would be 3.5, 20.7, and 51.9. Accordingly, the observed values are somewhat of the magnitude to be expected. On the whole, the absolute magnitude of values of X appears to be independent of the magnitude of Y , although, possibly, X increases slightly with Y .

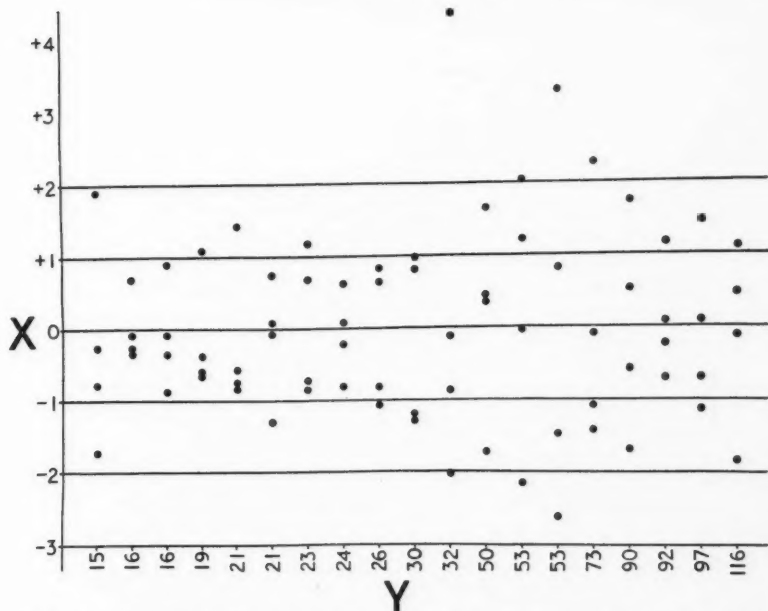


FIG. 1. Deviations of sub-plots of 1927 from plot expectation in terms of expected standard deviation.

The data from the four sub-plots, from which collections were made concurrently, support the hypothesis that if instead of any one value of n_s one had a series, they would tend to fluctuate about \bar{n}_s with a standard deviation of $\sqrt{\bar{n}_s}$. This finding suggests an estimate of variation for the value of flight as noted each evening. Using this estimate one can say whether the flight on any two successive evenings is significantly different.

Estimation of One Quantity from Observation of Another

Ability to estimate fluctuations in one quantity from a knowledge of fluctuations in another would be extremely useful in field work. For instance, if one knew the fluctuations from night to night in the catch in a light trap he might estimate the corresponding fluctuations in flight in a field of corn. Again, if one knew the latter fluctuations he might estimate fluctuations in rate of oviposition. From an understanding of the effect of meteorological conditions and of seasonal trend upon numbers of moths seen, one could estimate n_s from an observation of n_{s-1} .

Suppose one had observations on flight of moths over one plot and would estimate simultaneous flight, from evening to evening, over another plot. One supposes that the difference in fluctuation, from evening to evening, in observations over the two areas are mainly due to chance variations in the

numbers observed. One wishes to estimate the flight on the s^{th} evening in Plot II from the flight in the same evening on Plot I, if it is known that the flight on Plot II is λ times as great as that on Plot I. Call the flight on Plot I, n_{s1} , and the flight on Plot II, n_{s2} . Since one does not know \bar{n}_{s1} for the first plot, that is what the mean catch would have been if the observations could have been repeated indefinitely, but only n_{s1} , one must estimate on the basis of the latter quantity. On the basis of n_{s1} make an estimate of n_{s2} , namely \bar{n}_{s2} . As discussed above, chance variations in n_{s1} appear to form a Poisson distribution and to have a standard deviation of approximately $\sqrt{n_{s1}}$. If one may expect n_{s2} to be λ times greater than n_{s1} , the distribution of n_{s1} is stretched λ times to form the distribution of \bar{n}_{s2} , and the latter quantity would be calculated with a standard deviation of approximately $\lambda\sqrt{n_{s1}}$. The observation of n_{s2} may also be supposed to vary in a Poisson distribution with a standard error of approximately $\sqrt{n_{s2}}$. Therefore, the difference between n_{s2} and \bar{n}_{s2} should be approximately distributed with a standard deviation of $\sqrt{n_{s2} + \lambda^2 n_{s1}}$.

As an example of estimation of observations over one plot from those on another, estimates of flight over the laboratory plot in 1929 are made from the numbers of moths observed, concurrently, over the secondary plot of the same year. The estimates are compared with the observation actually made.

The laboratory plot is referred to as Plot II, and the secondary plot is referred to as Plot I. The catches from these two plots were shown, above, to be highly correlated. Since the total catch on laboratory plot is 108 and on the secondary plot is 248, $\lambda = \frac{108}{248}$. λ is used to calculate \bar{n}_{s2} from n_{s1} . A series of differences, $(n_{s2} - \bar{n}_{s2})$, is obtained. These quantities

TABLE V
COMPARISON OF OBSERVATIONS OVER ONE PLOT WITH THE ESTIMATES OF THESE OBSERVATIONS

Date	n_{s1}	$\lambda n_{s1} = \bar{n}_{s2}$	n_{s2}	$n_{s2} - \bar{n}_{s2}$	$\sqrt{\lambda^2 n_{s1} + n_{s2}}$	$\frac{n_{s2} - \bar{n}_{s2}}{\sqrt{\lambda^2 n_{s1} + n_{s2}}}$
July 4-6	6	2.6	5	+2.4	2.48	+1.0
July 7	22	9.6	14	+4.4	4.26	+1.0
July 8	21	9.1	8	-1.1	3.46	-0.3
July 9-11	32	13.9	7	-6.9	3.62	-1.9
July 12	24	10.5	12	+1.5	4.07	+0.4
July 13	24	10.5	3	-7.5	2.75	-2.7
July 14-15	15	6.5	5	-1.5	2.80	-0.5
July 16	10	4.4	5	+0.6	2.63	+0.2
July 17	43	18.7	14	-4.7	4.71	-1.0
July 18-21	7	3.0	3	0.0	2.08	0.0
July 22	12	5.2	6	+0.8	2.88	+0.3
July 23	11	4.8	6	+1.2	2.84	+0.4
July 24-25	8	3.5	5	+1.5	2.55	+0.6
July 26-27	5	2.2	7	+4.8	2.82	+1.7
July 29-31	8	3.5	8	+4.5	3.09	+1.5
	248		108			

should be approximately normally distributed with a standard deviation of, approximately, $\sqrt{n_{s2} + \lambda^2 n_{s1}}$. Since the catch on some days was very small, certain days were grouped. On this basis one obtains the series of calculations shown in Table V.

It can be seen that of the fifteen values of $\frac{n_{s2} - \bar{n}_{s2}}{\sqrt{\lambda^2 n_{s1} + n_{s2}}}$, one falls beyond ± 2 , 4.5 fall beyond ± 1 , and 9.5 fall within ± 1 . The corresponding figures from the normal curve would be 0.7, 4.1, and 10.2. Accordingly, it appears that the differences between n_{s2} and \bar{n}_{s2} are of about the magnitude to be expected by chance. The high degree of inaccuracy of the estimate of the n_{s2} , \bar{n}_{s2} , made upon n_{s1} , is obvious. Since the accuracy of \bar{n}_{s2} varies with the magnitude of n_{s1} , upon which it is based, far greater values of n_{s1} than those observed would be necessary to make such a method of much use.

Investigation of the Relation Between Numbers of Moths in Flight and Meteorological Conditions by the Variate-difference Method

As discussed elsewhere in this paper, the effect of seasonal trend upon numbers of moths in flight is marked and must be eliminated before the relation between these numbers and meteorological conditions can be determined. In order to eliminate the effect of seasonal trend from observations on numbers of moths in flight one may use the variate-difference method of correlation (3). This method was, of course, developed to eliminate just such influence of trend. While the variate-difference method furnishes a quantitative estimate of the relation between activity and meteorological conditions, it does not show the general nature of the relationship. The method is used in this paper to find whether there is a relation between number of moths in flight and evening temperature, daily maximum temperature, relative humidity, or wind velocity. Differences in physical conditions are examined against differences in catch for *Pyrausta nubilalis* Hubn.

The effect of evening temperature, which may be expected to be the most important meteorological condition affecting flight of moths, is first examined by the variate-difference method. For temperature of the evening, temperature at 30 minutes after sunset is used. An examination of the values obtained by correlating various differences of temperature and numbers of moths in flight is made with the purpose of finding what difference is the most satisfactory. The results obtained in this work are shown in Table VI.

TABLE VI
CORRELATION OF SUCCESSIVE DIFFERENCES OF NUMBERS OF MOTHS AND OF EVENING TEMPERATURE

Year	Date range, inclusive, of data	Raw numbers r_{tn}	First difference r_{tn}	Second difference r_{tn}	Third difference r_{tn}
1928	July 7 - Aug. 3	+ .23	+ .23	+ .18	+ .15
1929	July 5 - July 29	+ .36	+ .58	+ .56	+ .53
1930	June 30 - July 20	+ .15	+ .26	+ .29	+ .37
1931	July 3 - July 21	+ .06	+ .45	+ .45	+ .43
1932	June 29 - July 24	+ .18	+ .44	+ .53	+ .59
1933	June 24 - July 23	+ .50	+ .52	+ .46	+ .44

In general, the first difference showed a higher correlation than further differences. Accordingly, it is assumed that correlation of first differences is probably the most reasonable procedure. In the examination of the relation between numbers of moths and physical conditions, other than temperature, first differences are studied. One finds that values of $(n_s - n_{s-1})$ at the beginning and end of the season of flight, when seasonal trend may be supposed to be low, tend to be smaller than those obtained during the middle of the season. The smallness of these differences arises because the influence of seasonal trend has not been completely eliminated. Also, since it has been demonstrated that the magnitude of chance errors depends on the magnitude of flight and since the magnitude of flight depends, in part, on seasonal trend, then the magnitude of chance errors at the ends of the flight period must tend to be small. Accordingly, they must introduce chance variations of but small magnitude into differences of observations at these times. Further differences than the first are not profitable since the chance error of observations tends to be accentuated.

From Table VI it can be seen that in all years, and for all differences, the numbers of moths in flight and temperature are positively correlated. Therefore, it is certain that the number of moths taken increases with rise in temperature, in general.

The results for 1931 are of some interest. The correlation between temperature and numbers of moths, raw numbers, is very low, +0.06, and the correlation of first differences moderately high, +0.45. This result occurs because the temperatures at the end of the period of flight rose, as seasonal trend of flight fell. First differences, however, eliminated much of the effect of the trend.

Pyrausta nubilalis Hubn. flies in the evening when temperature is comparatively low. One may suppose, however, that the temperature during the warmer part of the day has an effect upon evening flight. Thus, the heat of the day may control the number of eggs matured and laid in the evening.

The relation between numbers of moths in flight and maximum temperature is examined in the same way as that between flight and evening temperature.

TABLE VII
CORRELATION OF FIRST DIFFERENCES OF MOTH NUMBERS
AND OF MAXIMUM TEMPERATURE

Year	Date range, inclusive, of data	r_{nj}	r_{nj-t}
1928	July 7 - Aug. 3	+ .28	+ .18
1929	July 4 - July 29	+ .47	+ .19
1930	July 1 - July 20	+ .05	- .06
1931	July 3 - July 19	.00	- .36
1932	June 29 - July 24	+ .25	- .22
1933	June 24 - July 23	+ .30	- .11

Since evening temperature and daily maximum temperature must be closely related, in addition to the correlation of number of moths in flight (n_s) and maximum temperature (j_s), a partial correlation is calculated, holding evening temperature (t_s) constant. The results are shown in Table VII.

The results from this examination show that flight is correlated positively with daily maximum temperature. However, such a relation does not exist when the partial correlation, holding evening temperature constant, is calculated. In this case one may say that there is not a significant relation between activity and maximum temperature, or else that linear relation is not adequate.

Humidity might have been expected to have a very great effect upon activity. The relation between humidity and numbers of moths in flight is examined in the same way as that between flight and maximum temperature. In addition to the simple correlation of numbers of moths in flight (n_s) and humidity (h_s), a partial correlation is calculated holding evening temperature (t_s) constant. Relative humidity at half an hour after sunset is used. The results are shown in Table VIII.

From the results of this examination one may conclude that humidity as measured, has, contrary to expectations, but little control over activity.

For wind (w_s), as for daily maximum temperature, and for humidity in the evening, correlation of first difference with first difference of numbers of moths in flight, and the partial correlation holding evening temperature constant, are calculated. Wind velocity at half an hour after sunset is used. The results are shown in Table IX.

The results from this examination show that wind has a depressing effect on flight. Since the correlation between evening temperature and wind velocity is low, the partial correlation between wind and numbers of moths, holding temperature constant, shows a clear-cut, negative relationship.

The depressing effect of wind upon flight must be very strong, since one finds such a marked negative relation between wind velocity readings for one hour and numbers of moths in flight for an evening. Wind velocity values taken thirty minutes after sunset are probably not a good index of wind velocities for the entire evening, since wind velocity changes rapidly

TABLE VIII
CORRELATION OF FIRST DIFFERENCES OF MOTH NUMBERS
AND OF HUMIDITY

Year	Date range, inclusive, of data	r_{hn}	$r_{hn.t}$
1928	July 7 - Aug. 3	-.14	-.02
1929	July 5 - July 29	-.22	-.39
1930	June 30 - July 20	+.04	+.11
1931	July 3 - July 21	+.43	+.33
1932	June 29 - July 24	-.16	-.18
1933	June 24 - July 23	-.16	.00

TABLE IX
CORRELATION OF FIRST DIFFERENCES OF MOTH NUMBERS
AND WIND VELOCITY

Year	Date range, inclusive, of data*	r_{wn}	$r_{wn.t}$
1928	July 7 - Aug. 3	-.22	-.22
1929	July 5 - July 29	-.21	-.16
1930	June 30 - July 20	-.32	-.33
1931	July 3 - July 21	-.51	-.51
1932	June 29 - July 24	-.34	-.29
1933	June 24 - July 23	-.36	-.30

*Less lacking observations, see Table II.

and erratically. No one value of wind velocity should be so typical of conditions for an evening as such a value of temperature. Temperature values taken soon after sunset should be a fairly good index of evening temperatures, since temperature changes comparatively slowly, and changes systematically.

One may sum up this examination of the relationship between numbers of moths and various physical factors by the variate-difference method, as follows. A powerful influence is exercised by evening temperature on numbers of moths, which increase with temperature. No clear-cut relation between activity and either maximum daily temperature or humidity is apparent. Wind velocity exercises a depressing effect upon flight.

Definition of Activity

The remainder of this paper is mainly concerned with the relation between activity of the corn borer moths and temperature. This relationship is investigated at some length since, as was shown above, the number of moths in flight is closely associated with evening temperature. Accordingly, it is useful to determine the relationship more closely than has been done. Also the methods of investigation may be useful for further work in determining other relations.

Since in the remainder of this paper the discussion is of activity under given conditions of evening temperature, the term "activity" should be defined. The activity of the moths is thought of as the extent to which moths came to the observation plot, compared with the extent to which they would have come under average temperature conditions. Accordingly, we take activity to be the ratio of the mean number of corn borer moths to the mean number which would be seen under average conditions of evening temperature, if observations could be repeated indefinitely. More exactly, we may say $\frac{\bar{n}_s}{D_s}$ is the activity, A_s , on the s^{th} evening. The value, \bar{n}_s , is as previously defined. The value, D_s , is the mean number of moths flying in a given observation plot under temperature conditions average for the period under examination, when an infinite number of such plots are observed.

In field work, as discussed in the introduction, one is mainly concerned with the relative activity obtaining between two given conditions. That is, one wishes to know how the catch under given conditions would compare with that under other conditions. The relative activity of the s^{th} and x^{th} evenings is $\frac{A_s}{A_x}$, or $\frac{\bar{n}_s}{D_s} / \frac{\bar{n}_x}{D_x}$. Such a value measures the relative suitability for flight of the s^{th} and of the x^{th} evenings.

Choice of Appropriate Seasonal Trend Lines

In finding the relation between activity and temperature, or other physical conditions, it is first necessary in some way to estimate seasonal trend of flight. The relation must be found by relating estimates of activity for each evening to the temperature conditions prevailing on that evening. From the nature of activity, as defined above, some estimate of the number of moths

to be expected under average temperature conditions must be made. It is shown below that the number of moths to be expected each evening, under average temperature conditions, varies with date of the evening. Accordingly, the effect of seasonal trend of flight must be estimated.

The magnitude of flight to be expected under any given temperature conditions varies greatly with the progress of the season of flight, since the entire season of flight of *Pyrausta nubilalis* Hubn. lasts only about five weeks. When flight starts, few moths are taken; for a week or so the numbers taken increase rapidly; later, the numbers decrease. This general seasonal trend of flight has occurred in observations made during seven years, in several fields, as can be seen in Table I.

In addition to the general impression obtained from observations on seasonal trend of corn borer moth flight, something of the nature of the trend may be learned from the life history of *Pyrausta nubilalis* Hubn., previously discussed. It is apparent that the larvae emerge from the winter at practically one level of development. Within any group subject to the same conditions there is variation in the time taken to reach the imaginal stage and in the duration of the imaginal stage. Further, one may expect a continuous variation in the rates of development in different situations. Moths from all these situations must tend to come to a given field, since the moths travel widely and freely. Altogether, one may expect a continuous variation in the abundance of moths in a given field. One may expect the flight to commence each season gradually, reach a peak, and gradually die away, as appears to be roughly the case.

It is of interest to note that, when data from the various years are fitted with fourth degree polynomial curves, but one peak in flight is obtained, except for the data of 1931 which show two peaks. Even in this case the bimodality is a temperature effect.

In order to fit a line to seasonal trends, biological workers have made use of a sliding average. A mathematical trend is more desirable in that one can readily estimate the significance of deviations from it.

Polynomials have been used widely in various fields of work to fit for trend against time. In the final form of analysis of the relation between activity and temperature, polynomials are used in this paper. This is done since polynomials are comparatively easy to fit and the calculation of summed deviations, squared, of observations about them is simple. Also, the function of activity in terms of temperature is solved as a polynomial. Accordingly, the functions of temperature and of time are made analogous.

In addition to the use of polynomials in fitting to seasonal trend, the use of frequency curves was tried. It appeared that when one knows the flight to have the characteristics discussed above, one can apply less general curves than polynomials. It was thought frequency curves should be applicable since the flight must approach zero asymptotically at the ends of its duration, and is most probably unimodal. In particular, the use of curves of the Pearson, or of the Gram-Charlier systems, was examined. For the reasons indicated above, the polynomial was ultimately chosen.

Estimation of Activity Relationships by Deviation from Trend Lines

It would, of course, appear that if one had fitted a line, even approaching the seasonal trend, one might take the deviations of observations from it as estimates of activity and relate these deviations to temperature. Such procedure was not, however, found feasible.

In the first place it should be pointed out that one does not want to fit for temperature conditions an equation of the same type and order as for date, and then try to relate the respective deviations. One wants a relation between activity and actual temperature conditions. If one knows such a relation he can apply the results in the field as soon as temperature observations are made. He can then estimate activity to be at a low, medium, or high level, according to physical conditions.

If one fit a seasonal trend line to observed data he does not obtain an estimate of the flight that would have occurred under average temperature conditions. He obtains, rather, a line taking into account only the values observed, and thus incorporating the effect of temperature. Thus, if for a period temperature was such as to depress or to inhibit flight, the trend goes down. However, during this period there might have been a great flight under average temperature conditions.

If one does fit a seasonal trend to the data of each year, and calls the trend value on the s^{th} night m_s , it would seem one might use, as the estimate of activity on the s^{th} night, the deviation of n_s from m_s . A good deal of work was done along this line. It was first thought to use $\frac{n_s - m_s}{m_s}$ as an estimate of activity to find the relation of activity to temperature conditions. However, for this estimate of activity, random error varies approximately with $\sqrt{n_s}$. If temperature conditions are fairly suitable for flight, n_s tends to vary with the magnitude of flight possible, *i.e.*, with the time of year and with the year. Accordingly, chance error tends to vary with the time of season and magnitude of seasonal flight. In order to avoid this difficulty of variable random error, use of the quantity $\frac{n_s - m_s}{\sqrt{m_s}}$ was made. In using this quantity one was faced with two difficulties. In the first place the random error does not vary as $\sqrt{m_s}$ but as $\sqrt{n_s}$. In the second place, one had an estimate of activity dependent on the time of season in part, not solely on activity.

On account of the difficulty in fitting a trend that was independent of temperature, and of the difficulty of using deviations from a trend line, the methods discussed above are not used in this paper.

Investigation by Comparing Successive Ratios for Flight with Changes in Temperature

Rather than attempt to estimate seasonal trend, and from it to attempt to estimate activity, one might choose an estimate of activity which is, to a certain extent, independent of trend. One can get such an estimate in the form of the quantity $\frac{n_s}{n_{s-1}}$, when studied in connection with t_s and with t_{s-1} . The

values, $(s - 1)$ and s , are subscripts used to denote two successive evenings on which data were collected.

The value $\frac{n_s}{n_{s-1}}$ furnishes an estimate of the relative activity between the temperatures t_s and t_{s-1} . The nature and value of estimates of relative activity are noted in the foregoing discussion on activity. Use of the quantity $\frac{n_s}{n_{s-1}}$ is justified as follows. If, as was previously supposed, the activity, A_s , on the s^{th} evening with temperature, t_s , equals $\frac{\bar{n}_s}{D_s}$, then, $\frac{n_s}{D_s}$ is an estimate of A_s . Similarly, $\frac{n_{s-1}}{D_{s-1}}$ is an estimate of A_{s-1} . The difference between the values of seasonal trend on successive evenings should be comparatively small. If D_s differs but little from D_{s-1} , one obtains an approximation to $\frac{A_s}{A_{s-1}}$ from $\frac{n_s}{n_{s-1}}$. Accordingly, in $\frac{n_s}{n_{s-1}}$ one obtains an estimate of the relative activity associated with the temperature conditions, t_s and t_{s-1} .

In Table X, values of $\frac{n_s}{n_{s-1}}$ are entered against t_s and t_{s-1} . In the compilation of this table, data on the flight of *Pyrausta nubilalis* Hubn. are used from the years 1928-1933, inclusive. Within these years the range of dates, inclusive, employed is as follows: in 1928 from July 7 to August 3, in 1929 from July 4 to July 30, in 1930 from June 29 to July 18, in 1931 from July 2 to July 19, in 1932 from June 28 to July 24, in 1933 from June 24 to July 22.

When both n_{s-1} and n_s are zero, $\frac{n_s}{n_{s-1}}$ has, of course, an indeterminate value. Such values have been represented in Table X by the sign \sim . When n_{s-1} is zero, and n_s is greater than zero, the value $\frac{n_s}{n_{s-1}}$ is, of course, infinite. Such values have been entered in the table as ∞ . Other values of $\frac{n_s}{n_{s-1}}$ have been entered as two-place decimals. A line has been drawn diagonally across the table through the region where $t_s = t_{s-1}$. Values to the left of this line, of course, occurred with falling temperatures and values to the right with rising temperatures. It may be pointed out that the quantities considered are total numbers of moths for each evening and temperature at thirty minutes after sunset.

From a table of such a quantity as $\frac{n_s}{n_{s-1}}$ one cannot get a numerical estimate of the degree of association between activity and temperature. However, Table X does show the characteristics of the relation between flight and temperature.

If the values in Table X are examined with respect to their distribution about the diagonal, where $t_s = t_{s-1}$, two tendencies are discernible. First, observations below and to the right of the diagonal tend to be greater than 1.00 and those to the left to be less than 1.00. It is shown below that there

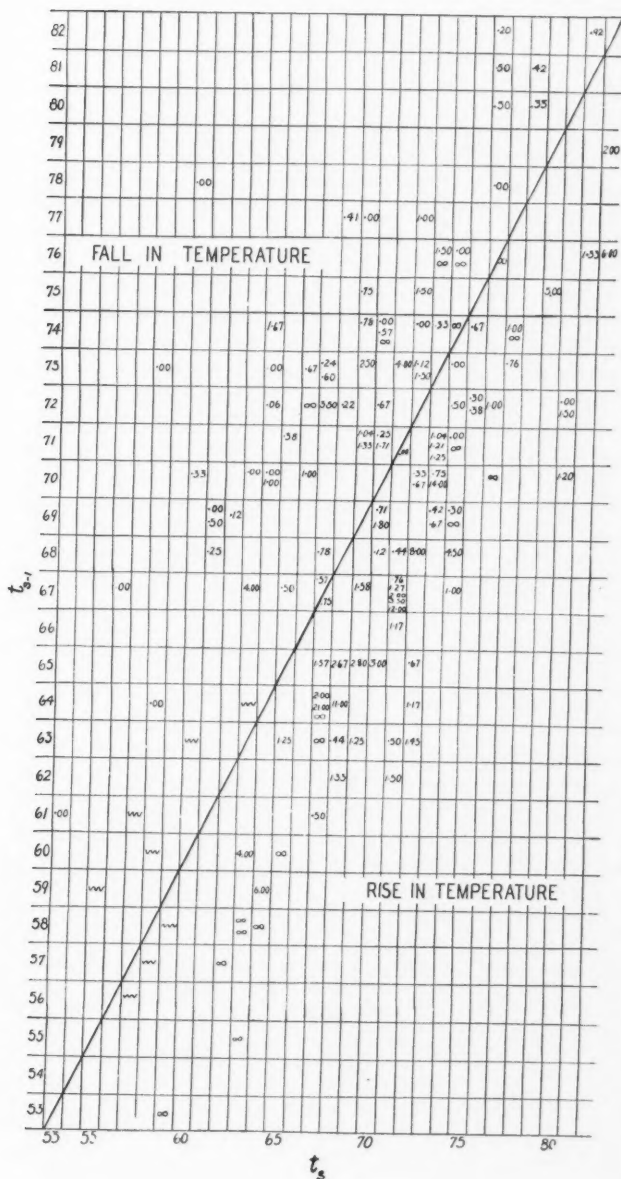
is a statistically significant tendency for values to be so arranged. Such a tendency means that relative activity falls when temperature falls and rises when temperature rises. Second, any two values of $\frac{n_s}{n_{s-1}}$, on any given perpendicular to the diagonal, and at equal distances from the diagonal, tend to be reciprocal. Throughout the field this tendency appears general. However, it is most intense in the region of the lower values of t_s and t_{s-1} . Such values of $\frac{n_s}{n_{s-1}}$ should be so reciprocal if there are given levels of activity associated with given temperatures. Suppose activity A_1 is associated with t_1 , and activity A_2 with t_2 , where $t_1 \neq t_2$. Then, the two values of $\frac{n_s}{n_{s-1}}$ are estimates of the values $\frac{A_1}{A_2}$ and $\frac{A_2}{A_1}$, respectively.

The significance of the apparent tendency for relative activity to fall and to rise with temperature may be investigated by determining the probability that the values in Table X could have been distributed as they were by chance. Two groups of values, those greater than, and those less than 1.00 are considered. Values of 1.00 are put half in each of these classes. In the upper right-hand part of the field, where t_s and t_{s-1} are 60° F. or more, there are 126 entries. Of these, 56 fall in the region where t_{s-1} is greater than t_s , that is, where temperature had fallen; 5 fall in the region where t_{s-1} equals t_s ; and 65 fall in the region where t_{s-1} is less than t_s , that is where temperature had risen. Of the 56 in the region where t_{s-1} is greater than t_s ; 17.5 are greater than 1.00; 36.5 are less than 1.00. The two indeterminate values are not considered. If the size of the ratios had been determined by chance and a ratio had as much chance to be over 1.00, as under, then one would expect 27, with a standard deviation of 3.67, in each group. The deviation of 9.5 from 27 is therefore significant. Therefore, the number of values less than 1.00 is significant. That is, fall in temperature reduces flight. Of the 65 in the region where t_{s-1} is less than t_s , 41.5 are greater than 1.00 and 23.5 less than 1.00. If the size of the ratios had been determined by chance and a ratio had as much chance to be more than 1.00, as to be less than 1.00, then one would expect 32.5, with a standard deviation of 4.03, in each group. The deviation of 9.0 from 32.5 is, therefore, significant. Therefore, the number of values greater than 1.00 is significant. That is, rise in temperature increases flight.

From Table X it can be seen that, as was reported by Stirrett (12), for temperatures less than 60° F. there can be no appreciable activity. Activity at any temperature above 60° F. is, with one exception, infinitely greater than that observed at temperatures below 60° F. Also, when t_s and t_{s-1} are below 60° F., indeterminate values are obtained.

A surface fitted to data of the type shown in Table X would furnish a most useful relation between activity and temperature. An unsuccessful effort was made to fit such a surface. In the first place, a satisfactory surface was

TABLE X
RATIO OF FLIGHT TO TEMPERATURE ON SUCCESSIVE EVENINGS



most difficult to find. In the second place, since the random error of the values $\frac{n_s}{n_{s-1}}$ was not normal, and was very great, these quantities were very difficult to use. These values may vary between 0.00 and $+\infty$ with half the values lying between 0.00 and $+1.00$. Some work on transformation of the values of $\frac{n_s}{n_{s-1}}$ was attempted, but it was not satisfactory.

Effect of Previous Temperature

Laboratory investigations by various workers show that rate of development of insects, at any given temperature, depends, in part, upon the temperature conditions to which the insect has been previously subject. Such work is reviewed by Uvarov (14). He says that "one point, however, is beyond dispute, namely, that fluctuations of temperature are not without an effect on the rate of development. This effect is often positive, particularly when a favorable temperature alternates with one below the zero of development (but not low enough to be injurious), while an alternation with high temperature is usually harmful." If one supposes rate of development and degree of activity to be similar phenomena, one would expect activity at a given temperature, when preceded by low temperatures to be different from, and probably greater than, that at the same given temperature preceded by high temperatures. However, little investigation seems to have been made of such possible modification of the relation between activity and current temperature. Accordingly, in this paper, an examination is made, below, of the possible modification.

In this paper an estimate of the relation between activity on a given night and temperature on that night and on the preceding night jointly is made. The solution must be joint, since the effect of previous temperature should not be shown directly but in the modification of the activity found with current temperature.

In the joint solution discussed above, only temperatures t_s and t_{s-1} are concerned. Of course, the response of moths to temperature on a given evening must be affected by more preceding temperatures than that of the evening immediately antecedent. However, the correlation between temperatures of successive evenings is so high, as shown below, that a great deal

TABLE XI
CORRELATION BETWEEN TEMPERATURES ON
SUCCESSIVE EVENINGS

Year	Date range, inclusive, of data	Correlation coefficient
1928	July 8 - Aug. 3	+ .62
1929	July 6 - July 29	+ .70
1930	July 1 - July 20	+ .63
1931	July 4 - July 21	+ .48
1932	June 30 - July 24	+ .62
1933	June 26 - July 23	+ .59

of information on preceding conditions is conveyed by the immediately antecedent evening, and probably little more information would be added by data from further preceding evenings.

The correlation between the temperatures of successive days is shown in Table XI.

Temperature values used are those obtaining half an hour after sunset. The range of data upon which the calculations are based is indicated.

Detailed Study of the Relation Between Activity and Evening Temperature

A detailed study of the relation between activity and evening temperature is made below. Above, the investigation by the variate-difference method showed flight and evening temperature to be consistently associated. Also, the investigation by comparing successive ratios for flight with changes in temperature showed the general nature of the relation between activity and evening temperature. In the preceding section, evidence was presented to show that one may expect the relation between activity and current temperature to vary with preceding temperature conditions.

In considering the joint effect of two or more factors upon flight of corn borer moth, it is not satisfactory to have the various effects additive. In the data under consideration, a single factor can be prohibitive of activity. Time of year can, obviously, be so prohibitive. In addition, low temperature, high wind and heavy rain may totally inhibit flight. If one should find certain functional relationships between numbers of moths and two or more factors, it would appear that the functions should be multiplied rather than added. By such an arrangement any factor can be prohibitive, and if one condition is optimal for activity, the activity varies with the other conditions. Accordingly, the form of joint solution suitable for temperature and time of year should consider the number of moths in flight the product of two functions, of date and temperature, respectively. As stated in the discussion on the nature of activity, $\bar{n}_s = D_s \cdot A_s$. If $D_s = f_1(d_s)$, where d_s is the date of the s^{th} day reckoned from an arbitrary and convenient origin, and $A_s = f_2(t_s)$, then $\bar{n}_s = f_1(d_s) \cdot f_2(t_s)$. Approximately, $n_s = f_1(d_s) \cdot f_2(t_s)$. The multiplicative nature of the effect of various factors appears to have been most clearly recognized by workers on the manurial value of various compounds. Thus Stewart (11) quotes the formula,

$$y = A(1 - e^{-cx})(1 - e^{-c_1x_1}) \dots (1 - e^{-c_nx_n})$$

where y is observed yield, A is maximum yield, and each other factor to the right is the effect of some one manure. The values, c, c_1, \dots, c_n are constants to be determined and x, x_1, \dots, x_n are quantities of n different fertilizers.

The relations between activity and date must be determined separately for each year for two reasons. First, the shape and amplitude of the date relation probably varies in various years. Second, even if the shape and amplitude of the function of date were essentially the same from year to year, and hence capable of being represented by the same function, the flight occurs at different times from year to year and it is very difficult, since the date trend is obscured by the temperature relation, to decide what are the corresponding dates in different years.

The relation between activity and temperature must be determined jointly for all the years. The procedure of combining the data from the years seems to be fundamentally sound, since one would suppose the reactions of the moths to environmental conditions in any year to be a constant, specific character. Also, it would be difficult to fit a relationship with many constants from the data of a single year since in any one there were only about thirty nights when flight might occur.

For the determination of the relations between numbers of moths and, date and temperature, respectively, the methods of (7) and (6) were not satisfactory. By the method of (7) the effect of the various physical factors would necessarily be additive, whereas, in this problem, they are manifestly multiplicative. Also, there is no apparent way of combining the data from various years. In the method of Janisch the work hinges on a determination, under experimental conditions, of the temperature optimal for activity. In the field one cannot make such a determination.

A simultaneous solution for $f_1(d_s)$ and for $f_2(t_s)$ in the equation

$$n_s = f_1(d_s) \cdot f_2(t_s)$$

is impossible. It is impossible because one must solve for the relation between activity and date independently for each year, and for the relation between activity and temperature collectively for all years. Accordingly, solutions are made separately for the two relations. First, neglecting function of temperature, an approximation to function of date is found for each year. On the basis of this approximation to function of date, a first approximation to function of temperature, for combined years, is found. Second approximations to the functions of date and temperature are found by approximating to one relation upon the basis of the earlier approximation to the other relation.

The solution for $f_1(d_s)$ is a polynomial,

$$a_0 + a_1 d_s + a_2 d_s^2 + a_3 d_s^3 + a_4 d_s^4$$

where a_0, a_1, a_2, a_3 and a_4 are constants to be found. An equation of the fourth degree is considered adequate since, as was discussed in the section on choice of appropriate trend lines, one may expect flight to have only one peak in the course of a season. The solution for $f_1(d_s)$ is made from the equation

$$n_s = (a_0 + a_1 d_s + a_2 d_s^2 + a_3 d_s^3 + a_4 d_s^4) A_s$$

where A_s is the activity, as calculated from an approximation to the relation between flight and temperature on the s^{th} day.

In the calculation of the first approximation to $f_1(d_s)$ all values of A_s are assumed equal to one, that is, the effect of temperature is neglected. For each year an equation

$$n_s = a'_0 + a'_1 d_s + a'_2 d_s^2 + a'_3 d_s^3 + a'_4 d_s^4$$

where $a'_0 \dots a'_4$ are the coefficients of the first approximation, is fitted

by the method of least squares. In the second approximation, for each year an equation

$$n_s = (a_0'' + a_1''d_s + a_2''d_s^2 + a_3''d_s^3 + a_4''d_s^4) A_s'$$

where $a_0'' \dots a_4''$ are coefficients of the second approximation, and where A_s' is the approximation to the activity on the s^{th} evening as calculated from the first approximation to the relation between activity and temperature is fitted. The values for the approximations to $f_1(d_s)$ are not shown in this paper.

The approximations to $f_1(d_s)$ are based upon prior estimates of the activity, A_s , of each evening. For each evening one is fitting a trend value, adjusted for the appropriate activity. It is from the modified values of the trend chosen that the observations deviate to a minimum extent. Thus, if temperature is such as to inhibit flight the trend value is adjusted to a zero and the deviation of the observation is zero. Accordingly, temperature conditions, for an evening or for a period, do not affect fit of the seasonal trend. Similarly, approximations to $f_2(t_s)$ are based upon prior estimates of the seasonal trend value, D_s , of each evening. One is, essentially, trying to find a temperature relation which will so modify the date trend that observations shall deviate from the modified values to a minimum extent.

Date trends and the relation between activity and temperature are determined from the data of the years 1928 to 1933, inclusive. The data of 1927 are omitted because they are not complete for the year, and because the methods and general volume of catch differ radically from methods and volume in other years. The data used in the calculation of approximations to $f_1(d_s)$ cover the following dates, inclusive, in the various years: in 1928 from July 6 to August 11, in 1929 from July 3 to August 2, in 1930 from June 28 to July 26, in 1931 from June 29 to July 21, in 1932 from June 27 to July 26, in 1933 from June 23 to July 24. The curve obtained, in fitting a polynomial to date, varies according to the number of days, beyond the range of observed flight, which are included in the fitting. The ranges of dates, shown above, are chosen so that there be one evening with no flight before the first observed flight and two such evenings after the last observed flight, in each season.

The fourth degree polynomials fitted for the relation between numbers of moths in flight and date give curves with but one peak of flight, except for the data of 1931, which curve has two peaks. This effect appeared to be due to the inhibitive effect of cool temperatures about July 10 and 11, since on the second fitting when temperature effects are largely eliminated, there is only one peak.

In determining the relation between activity and temperature it would appear desirable to start with some very general curve. Relationships such as, for instance, exponentials, or curves involving steady geometrical increase of activity, appear undesirable. Accordingly, suppose that the relation may be represented by a polynomial.

A polynomial of the third degree should be adequate for the relation, since one may suppose there is only one region in the temperature range optimal

for activity. Accordingly, one may suppose activity of moths on any day varies with temperature on that day in the following manner:

$$A_s = b_0 + b_1 t_s + b_2 t_s^2 + b_3 t_s^3 \quad (1)$$

where b_0, b_1, b_2, b_3 , are constants to be determined. Although, as was suggested above, the relation between activity and current temperature may be expected to be modified by temperature on the preceding evening, this modification is neglected for the moment.

Examination of Table X shows that no appreciable activity occurs at temperatures of 60° F. or lower. Knowing this, one may restrict the polynomial so that activity for $t_s = 60^\circ \text{F.}$ is zero, and assume activity at all lower temperatures zero. If 60° F. is chosen as the origin of t_s and all fitting below 60° F. is neglected, Equation (1) becomes,

$$A_s = b_1 t_s + b_2 t_s^2 + b_3 t_s^3 \quad (2)$$

Equation (2) is constructed on the assumption that the relation of activity to temperature is constant from day to day. There is, however, as discussed above, good reason to suppose the relation varies with the temperature of the preceding evening. If the relation between temperature and activity varies continuously as the temperature of the previous evening varies, then each of the constants in Equation (2) must so vary. Accordingly, suppose,

$$b_1 = c_2 + c_3 t_{s-1}, \quad b_2 = c_4 + c_5 t_{s-1}, \quad \text{and} \quad b_3 = c_6 + c_7 t_{s-1}$$

where c_2, \dots, c_7 are constants to be determined. Substituting for b_1, b_2, b_3 , in equation (2) one gets

$$A_s = c_2 t_s + c_3 t_s t_{s-1} + c_4 t_s^2 + c_5 t_s^2 t_{s-1} + c_6 t_s^3 + c_7 t_s^3 t_{s-1}. \quad (3)$$

Equation (3) is referred to as $f_2(t_s)$.

From Equation (3) one can find the relation between activity and temperature on the s^{th} , or $(s-1)^{\text{th}}$ evenings. For t_s constant, one obtains an equation of the form

$$A_s = (c_2 t_s + c_4 t_s^2 + c_6 t_s^3) + (c_3 t_s + c_5 t_s^2 + c_7 t_s^3) t_{s-1}$$

where activity varies with t_{s-1} in the form, $A_s = k_0 + k_1 t_{s-1}$. The quantities k_0 and k_1 vary as third order equations in t_s . For t_{s-1} constant the variation of activity is a third degree polynomial in t_s .

In the calculation of the values of the coefficients in Equation (3), data from evenings when temperature was below 60° F. are not included. Such evenings are omitted, since in them activity has been assumed to be zero. Including such evenings, the data used in the calculations of approximations to $f_2(t_s)$ cover the following periods in the various years: in 1928 from July 8 to August 1, in 1929 from July 3 to July 31, in 1930 from June 29 to July 19, in 1931 from June 30 to July 20, in 1932 from June 29 to July 20, in 1933 from June 24 to July 22.

The constants, c_2, c_3, \dots, c_7 , of Equation (3) are calculated from the data by the method of least squares. The first approximation to $f_2(t_s)$ was calculated as a second degree polynomial in t_s , that is, with four terms, and next as a third degree polynomial in t_s , that is, with six terms. The curves and the deviations from the curves from the lower order solution were very similar to those from the higher. The higher order solution was chosen for calculating the second approximation to $f_1(d_s)$ in each year. Such a choice was not justifiable on the basis of improvement of fit; the choice was made in order to have the first and second approximations to $f_2(t_s)$ homologous. It appeared probable that a six term solution might be more suitable than a four term for the calculation of the second approximation.

As discussed above, an approximation to $f_1(d_s)$ is first calculated, and next, on this basis, an approximation to $f_2(t_s)$. The equation obtained in the first approximation to $f_2(t_s)$ is

$$n_s = +.2092 t_s D'_s - .008,715 t_s t_{s-1} D'_s - .005,004 t_s^2 D'_s \\ + .000,307,7 t_s^2 t_{s-1} D'_s + .000,046,12 t_s^3 D'_s - .000,002,3 t_s^3 t_{s-1} D'_s$$

where D'_s is the first approximation to D_s , the mean number of moths flying under average temperature conditions, with infinite observation, over the plot. The value D'_s is obtained from the first approximation to $f_1(d_s)$.

The general nature of this solution for $f'_2(t_s)$ is indicated in Fig. 2. The relation between t_s , temperature in degrees Fahrenheit in excess of 60°F .,

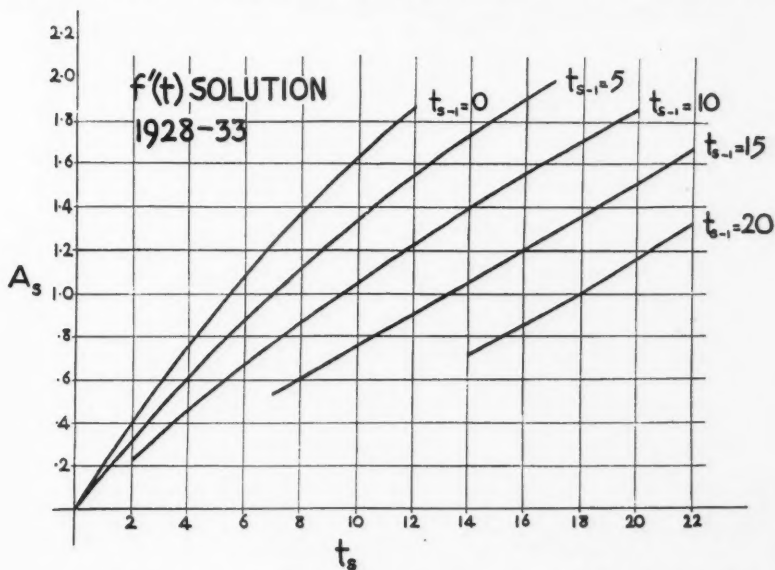


FIG. 2. Solution for the relation between temperature in excess of 60°F . and activity, first approximation. Data 1928 to 1933 inclusive.

and activity, with t_{s-1} constant, is plotted for t_{s-1} at intervals of five degrees Fahrenheit. Values of A'_s necessary for the calculation of $f''_1(d_s)$ are found. These values are not tabled. They are, however, similar to those tabled for $f''_2(t_s)$, which are shown below.

The activity found under average temperature conditions receives in the calculation of $f_2(t_s)$ the value of 1.00. Of course, the average is weighted by the number of moths to be expected from the date trend. All other values of activity are referable to this value.

Upon the basis of the second approximation to $f_1(d_s)$, a six-term, second approximation to $f_2(t_s)$ is calculated. The equation obtained is

$$n_s = +.160,861 t_s D''_s + .003,201,29 t_s t_{s-1} D''_s + .005,905,40 t_s^2 D''_s \\ - .001,567,61 t_s^2 t_{s-1} D''_s - .000,504,613 t_s^3 D''_s + .000,068,168,7 t_s^3 t_{s-1} D''_s$$

where D''_s , the second approximation to D_s , is obtained from the second approximation to $f_1(d_s)$.

When Equation (3) is put in the form

$$n_s = (c_2 + c_3 t_{s-1}) t_s + (c_4 + c_5 t_{s-1}) t_s^2 + (c_6 + c_7 t_{s-1}) t_s^3$$

and t_{s-1} is chosen any constant value, an equation for n_s in terms of t_s , of the type

$$n_s = b_1 t_s + b_2 t_s^2 + b_3 t_s^3$$

is obtained. Such coefficients, b_1 , b_2 , and b_3 , are calculated for all values of t_{s-1} from 57° F. to 82° F., inclusive. From the twenty-six resulting equations values of the activity for various values of t_s and t_{s-1} are calculated. These values are shown in Table XII.

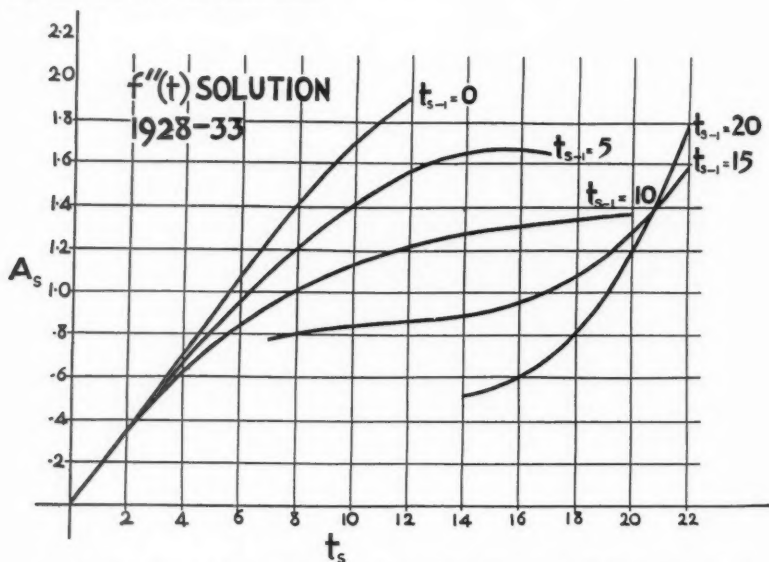


FIG. 3. Solution for the relation between temperature in excess of 60° F. and activity, second approximation. Data 1928 to 1933 inclusive.

TABLE XII
SECOND APPROXIMATION TO $f_2(t_s)$

t_s	t_{s-1}																									
	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82
60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00															
61	.16	.16	.16	.17	.17	.17	.17	.17	.17	.18	.18	.18	.18													
62	.34	.34	.34	.34	.34	.34	.34	.34	.35	.35	.35	.35	.35	.35												
63	.53	.53	.52	.52	.52	.51	.51	.51	.51	.51	.50	.50	.50	.50	.49											
64	.73	.72	.71	.71	.70	.69	.68	.67	.67	.66	.65	.64	.63	.63	.62	.61										
65	.93	.92	.90	.89	.87	.86	.84	.83	.82	.80	.79	.77	.76	.74	.73	.71	.70									
66	1.14	1.11	1.09	1.07	1.05	1.02	1.00	.98	.96	.93	.91	.89	.87	.84	.82	.80	.78	.75								
67	1.34	1.30	1.27	1.24	1.21	1.18	1.15	1.12	1.09	1.06	1.03	.99	.96	.93	.90	.87	.84	.81	.78							
68	1.49	1.45	1.41	1.37	1.33	1.29	1.25	1.21	1.17	1.13	1.09	1.05	1.01	.97	.93	.89	.85	.81	.77							
69	1.66	1.61	1.56	1.51	1.46	1.41	1.36	1.32	1.27	1.22	1.17	1.12	1.07	1.03	.98	.93	.88	.83	.78	.77						
70		1.75		1.69	1.64	1.58	1.52	1.47	1.41	1.36	1.30	1.24	1.19	1.13	1.07	1.02	.96	.90	.85	.79	.73	.67				
71			1.81	1.75	1.68	1.62	1.56	1.49	1.43	1.37	1.30	1.24	1.18	1.11	1.05	.98	.92	.86	.79	.73	.67	.61	.59			
72				1.91	1.84	1.77	1.70	1.63	1.56	1.49	1.42	1.35	1.28	1.21	1.14	1.07	1.00	.94	.87	.80	.73	.66	.58	.59		
73					1.91	1.83	1.76	1.69	1.61	1.54	1.47	1.39	1.32	1.25	1.17	1.10	1.02	.95	.88	.80	.73	.66	.58	.58		
74						1.87	1.80	1.72	1.65	1.57	1.50	1.42	1.35	1.27	1.20	1.12	1.04	.97	.89	.82	.74	.67	.59	.52		
75							1.81	1.74	1.67	1.59	1.52	1.44	1.37	1.29	1.22	1.14	1.07	.99	.92	.84	.77	.70	.62	.55		
76								1.74	1.66	1.59	1.52	1.45	1.38	1.31	1.24	1.17	1.10	1.03	.96	.88	.81	.74	.67	.60	.53	
77									1.64	1.58	1.52	1.45	1.39	1.33	1.26	1.20	1.13	1.07	1.01	.94	.88	.82	.75	.69	.62	.56
78										1.55	1.50	1.44	1.39	1.34	1.29	1.23	1.18	1.13	1.08	1.02	.97	.92	.86	.81	.76	.71
79											1.43	1.39	1.35	1.31	1.28	1.24	1.20	1.16	1.13	1.09	1.05	1.01	.98	.94	.90	
80												1.43	1.39	1.35	1.31	1.28	1.24	1.22	1.21	1.19	1.17	1.15	1.13	1.11	1.09	
81													1.37	1.35	1.33	1.31	1.30	1.28	1.26	1.24	1.22	1.21	1.19	1.17	1.15	
82														1.40	1.40	1.41	1.41	1.42	1.43	1.44	1.45	1.46	1.47	1.48	1.81	

All values of $f_2(t_s)$ for $t_s < 60^\circ F.$ are 0.00.

From Table XII the relation between t_s and activity is plotted in Fig. 3, for t_{s-1} constant at intervals of five degrees Fahrenheit. Figs. 2 and 3 are comparable. The second approximation to $f_2(t_s)$ gives very similar curves to the first approximation.

In order to evaluate a calculated relation, deviations, squared, of observations about the relation are summed. This sum is compared with the summed deviations squared about the mean of the observations, in each year, over the range of dates used in calculating the relation. The reductions in sums effected by the two approximations to the relation $f_1(d_s)$ are first compared. Secondly, the reductions effected by the approximations to $f_2(t_s)$ are compared.

The quantities given in Table XIII, as the sums of squares about the approximations to the date relations in each year, are readily calculated from the summations made in fitting the relations. The sum of squares about the first approximation to the relations is

$$\sum_s n_s^2 - a'_0 \sum_s n_s - a'_1 \sum_s n_s d_s - a'_2 \sum_s n_s d_s^2 - a'_3 \sum_s n_s d_s^3 - a'_4 \sum_s n_s d_s^4.$$

The sum of squares about the second approximation to the relation is

$$\sum_s n_s^2 - a''_0 \sum_s n_s A'_s - a''_1 \sum_s n_s d_s A'_s - a''_2 \sum_s n_s d_s^2 A'_s - a''_3 \sum_s n_s d_s^3 A'_s - a''_4 \sum_s n_s d_s^4 A'_s.$$

TABLE XIII

SUMMED DEVIATIONS SQUARED ABOUT APPROXIMATIONS TO $f_1(d_s)$ AND VARIANCE FOR EACH YEAR

Year	Days in range of dates	Sum of squares about mean	Sum of squares about $f_1(d_s)$	Sum of squares about $f_1^2(d_s)$
1928	37	1702.0	889.6	685.5
1929	31	465.7	307.2	214.2
1930	29	3750.1	2803.3	2320.6
1931	23	224.6	143.5	117.8
1932	30	1319.0	590.2	339.0
1933	32	3774.2	1783.4	679.7

It is apparent that both approximations are profitable, since in all years the sum of deviations, squared, about the first approximation to the function is much smaller than the variance. The corresponding sums for the second approximation are all a little smaller than those for the first approximation. It should, however, be noted that the second approximation to the function of date is made upon, and including, the first approximation to the function of temperature.

In the same way as the reduction in sums of deviations squared was calculated about the functions of date, so are sums of deviations squared calculated about the functions of temperature. The results are as shown in Table XIV. The range of dates upon which temperature calculations are based is slightly shorter than that used in the calculations on date. The sum

of squares about the first approximation to the relation is:

$$\sum_s n_s^2 - c'_2 \sum_s n_s t_s D'_s - c'_3 \sum_s n_s t_s t_{s-1} D'_s - c'_4 \sum_s n_s t_s^2 D'_s - c'_5 \sum_s n_s t_s^2 t_{s-1} D'_s \\ - c'_6 \sum_s n_s t_s^3 D'_s - c'_7 \sum_s n_s t_s^3 t_{s-1} D'_s.$$

The sum of squares about the second approximation to the relation is

$$\sum_s n_s^2 - c''_2 \sum_s n_s t_s D''_s - c''_3 \sum_s n_s t_s t_{s-1} D''_s - c''_4 \sum_s n_s t_s^2 D''_s - c''_5 \sum_s n_s t_s^2 t_{s-1} D''_s \\ - c''_6 \sum_s n_s t_s^3 D''_s - c''_7 \sum_s n_s t_s^3 t_{s-1} D''_s.$$

TABLE XIV

SUMMED DEVIATION SQUARED ABOUT APPROXIMATION TO $f_2(t_s)$ AND VARIANCE, FOR EACH YEAR

Year	Days in range of dates	Sum of squares about mean	Sum of squares about $f_2(t_s)$	Sum of squares about $f_2''(t_s)$
1928	25	1378.0	728.3	654.0
1929	29	439.8	313.9	215.4
1930	21	3405.0	2612.8	2177.9
1931	21	203.1	95.4	121.7
1932	22	1028.6	299.5	269.5
1933	29	3432.8	717.9	380.5

It is apparent that the first approximation to $f_2(t_s)$ greatly reduces the sum of deviations squared. The second approximation effects some reduction over the first in data of all years, except of 1931.

From these data on corn borer moths it is apparent that activity increases, in general, with current temperature. The activity associated with any current temperature is affected by preceding temperatures. The rate at which activity increases with increase in current temperature depends upon preceding temperature. There are indications that the rate at which activity increases with rise in temperature tends to decrease when temperature becomes very high relative to that of the preceding night. The temperature at which activity is a maximum depends upon previous temperature conditions. The existence and general nature of the temperature effects conform with findings (14) on the effect exerted by change in temperature upon rate of development.

The labor involved in making the fits by least squares, for two approximations to $f_1(d_s)$ and $f_2(t_s)$, was great. The method of solution used was that of Gauss (1). Use of this method reduced the work a great deal.

It is desirable to see how closely the values estimated from $f_1''(d_s)$ and $f_2''(t_s)$ agree with the observed magnitude of flight. Let \bar{n}_s be, as previously, the estimate of flight over the laboratory plot on the s^{th} evening, and $\bar{n}_s = f_1''(d_s) \cdot f_2''(t_s)$. In Fig. 4, magnitude of the values of \bar{n}_s are shown as narrow, solid columns, and of n_s as broad hollow columns. In calculating values of \bar{n}_s a few cases occurred where the values, t_s, t_{s-1} , were one or two degrees outside the range of Table XII. In these cases values, $f_2''(t_s)$, were obtained by extrapolation. However, for July 31, 1929, when t_{s-1} was 66° F. and

t_s was 80°F. , no value for $f_2''(t_s)$ could be found. Except for the deletion of this abnormal case, the limits of the range of dates, considered for each year in Fig. 4, are those used in finding $f_2''(t_s)$. In the figure the limits of the period are indicated by thin vertical lines.

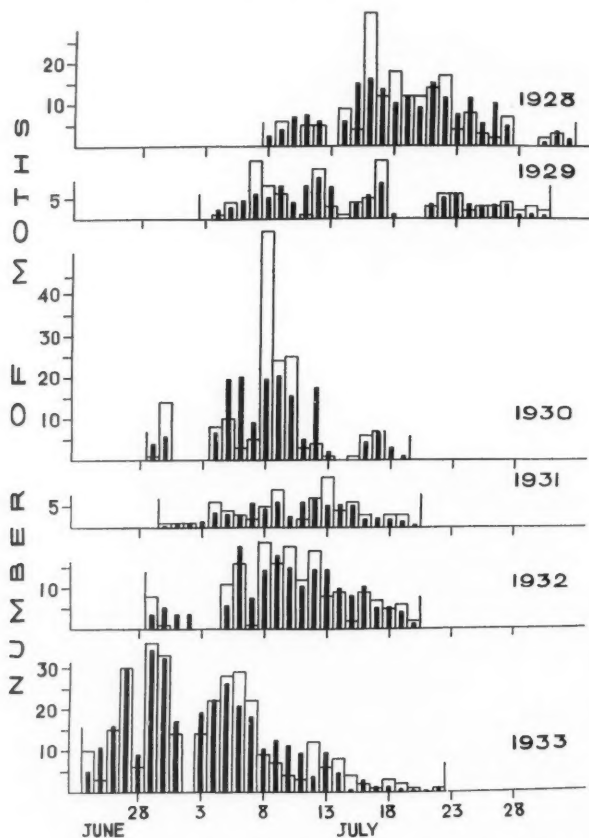


FIG. 4. Observed flight, in hollow columns, and values estimated from date and temperature relation, in solid columns.

From Fig. 4, it can be seen that there is general agreement between the magnitude of \bar{n}_s and n_s . There is, however, also considerable variation between the estimated and observed values. The situation is examined more closely in Table XV. In this table the estimated values are grouped for ranges of one moth and against each range observed values are entered. For ranges with more than one entry, mean, \bar{x} , and variance, s^2 , of the observations are calculated.

From Table XV, it can be seen that in each range the mean of the observed values varies closely with the magnitude of the estimated value. Also, the variance in each range varies, as was anticipated in the preceding section on the chance error of observations, with the magnitude of the estimated value. In that section it was shown that the variance, if ascribable only to random variability, would be approximately equal to the estimated value. In fact, there are two sources of variability, random variability and the effect of conditions other than date and temperature, within each range. Accordingly, the variance is larger than estimated values in Table XV. The proportional difference between estimated value and variance is greatest when the estimated value is great, for then temperature conditions tend to be optimal and, from the multiplicative nature of the joint effect of various factors, other physical conditions must effect flight greatly.

Conclusions

It is found that, on a given evening, variation in numbers of moths over an area is distributed with the standard deviation of these chance variations equal to the square root, approximately, of the observation. This relationship should be of use in handling data from such sources as surveys or traps. Thus, one should be able, neglecting seasonal trend, to judge whether observations made on successive evenings differ significantly. Since this preliminary study shows flight of corn borer moths to fluctuate in a nearly parallel manner over various areas, it is reasonable to seek causes, common to a district, of fluctuations.

From the relation between flight of the corn borer moth and temperature, the importance of weather during a season may be estimated and the probable success in extension of geographical distribution forecast. The relationship shows that there is, within the temperature range where flight is possible, so much adaptability in response, on the part of the moths, that it is improbable, provided temperature is not consistently very low, that paucity of moths is ascribable to the effect of temperature on flight. The relationship, also, foreshadows the success of the borer as it spreads, in North America, west and south. On the one hand, there is no ground for hope that the high night temperatures of the southern Mid-west will prove detrimental to flight of the moth. On the other hand, cool evenings in the high altitudes of the West and in the neighborhood of the Pacific Ocean, will act against the species.

An examination was made of the correspondence, each night, between magnitude of flight, as observed, and as estimated from the determined relations of flight to date and to temperature. For narrow ranges of estimated values the mean of the observed values varied closely with the magnitude of the estimated value. Also, the variance in each range varied with the magnitude of the estimated value. The effect of conditions other than date and temperature was greatest when the estimated value was great, for then temperature conditions tended to be optimal and, from the multiplicative nature of the joint effect of various factors, physical conditions other

than temperature affected flight greatly. This examination indicates that an estimation of flight over a small area is necessarily inexact on account of chance variation. However, for a wider area the favorableness of temperature conditions may be estimated.

The methods used in studying flight of the corn borer moth may be applied in studying activities of other species of insects. Such application would determine the importance of various physical conditions. Under what conditions a given activity may be expected to be great, or in what new regions the insects may succeed, could be judged.

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